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March, 1908.

THE STANDARDIZATION OF TETANUS ANTITOXIN

(AN AMERICAN UNIT ESTABLISHED UNDER
AUTHORITY OF THE ACT OF JULY 1, 1902)

BY

M. J. ROSENAU
and
JOHN F. ANDERSON.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1908.

TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.

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Biological
& Medical
Serials

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THE STANDARDIZATION OF TETANUS ANTITOXIN[a]

[*An American unit established under authority of the act of July 1, 1902.*]

By Milton J. Rosenau,
Surgeon, Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service,
and
John F. Anderson,
Passed Assistant Surgeon, Assistant Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

INTRODUCTION.

There are now four methods of measuring the strength of tetanus antitoxin: (1) The German method described by Behring, (2) the French method of Roux, (3) the Italian method after Tizzoni, and (4) the American method described in this bulletin.

The European standards are admitted to be unsatisfactory and, for the most part, not accurate; further, they are complicated and difficult to carry out. The American method adopted officially under the law of July 1, 1902, for this country is the result of several years, work upon this subject in the Hygienic Laboratory and it is believed will commend itself for its simplicity, directness, and accuracy.

The standard toxines and antitoxins are preserved under special precautions to prevent deterioration and are tested against each other reciprocally, so that the least alteration in either may be detected.

While the unit is based upon the neutralizing value of an arbitrary quantity of antitoxic serum, the *antitoxin* is not issued to other laboratories for the purposes of test, as is the case with diphtheria. A stable precipitated tetanus *toxine*, the test dose of which has been carefully determined, is given out. All the tetanus antitoxic serums for use in man upon the American market are now measured against this same standard toxine and have therefore precisely comparable strengths.

The need of uniformity in standardizing this valuable prophylactic serum is evident from the table on page 11, from which it will be seen

---

[a] Manuscript submitted for publication March 4, 1908.

that before the establishment of an American standard the tetanus serums upon the market varied extravagantly in the unit strength claimed, and were, for the most part, comparatively weak in true antitoxic potency. Since the promulgation of this standard unit the serums on the market have decidedly greater antitoxic value. The feebleness of the foreign serums compared with those of American manufacture is also evident from the table.

The object of this bulletin is to describe in detail the methods used in preparing and using this standard. A discussion of the theoretical considerations is introduced for a better understanding of the principles involved.

AMERICAN UNIT, AND METHODS OF STANDARDIZATION.

DEFINITIONS.—"The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine-Hospital Service."

The unit was thus officially defined October 25, 1907, in Treasury Department Circular No. 61. This circular amended the regulations promulgated in accordance with the act approved July 1, 1902, entitled "An act to regulate the sale of viruses, serums, toxins, and analogous products in the District of Columbia, to regulate interstate traffic in said articles, and for other purposes."

Based largely upon the work on the standardization of tetanus antitoxin done in the Hygienic Laboratory, a special committee of the Society of American Bacteriologists, which met in New York December 27 and 28, 1906, made the following report, which was unanimously adopted:

> That tetanus antitoxin be standardized by the tetanus toxin furnished by the Public Health and Marine-Hospital Service. The unit is ten times the least amount of serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of the standard toxin. The test dose is 100 minimal lethal doses of a precipitated toxin preserved under special conditions at the Hygienic Laboratory of the Public Health and Marine-Hospital Service. It was decided that the minimal immunizing dose for a case of possible infection through a wound should be 1,500 of such units. It was decided that after April 1 the new unit should be adopted by all producers of tetanus antitoxin.
>
> J. J. KINYOUN, *Chairman.*
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> JOSEPH MCFARLAND.
> M. J. ROSENAU.
> WILLIAM H. PARK, *Secretary.*

The American unit for measuring the strength of tetanus antitoxin may be defined as the neutralizing power possessed by an arbitrary quantity of antitetanic serum preserved under special conditions to prevent deterioration in the Hygienic Laboratory of the Public Health and Marine-Hospital Service. This arbitrary quantity now contains ten times the amount of tetanus antitoxin necessary to neutralize somewhat less than 100 minimal lethal doses of a standard toxine for a 350-gram guinea pig—that is, one-tenth of a unit mixed with 100 minimal lethal doses of the standard toxine contains just enough free poison in the mixture to kill the guinea pig in four days after subcutaneous injection.

The standardization of tetanus antitoxin does not differ radically from the standardization of diphtheria antitoxin. The toxines and antitoxins are measured against each other reciprocally, so that change or deterioration of either the standard toxine or the standard antitoxin may readily be determined. Duplicate toxines and antitoxins made from time to time will act as checks against deterioration of either the standard toxines or antitoxins.

The value of an unknown serum is measured indirectly through the toxine, using the L+ dose as the test dose. The L+ dose is the smallest quantity of tetanus toxine that will neutralize one-tenth of an immunity unit, plus a quantity of toxine sufficient to kill the animal in just four days.

The *toxine* and not the *antitoxin* is given out to licensed manufacturers and others interested for the purposes of standardizing their serums. The L+ or test dose of the particular toxine (A) now dispensed contains just 100 minimal lethal doses for a 350-gram guinea pig. This particular toxine is very stable and has not changed appreciably in two years. As soon as it alters or is exhausted the next toxine that will be issued may contain considerably more or less than 100 minimal lethal doses, but the test dose will contain precisely the same neutralizing power.

The antitoxic serum, for the purposes of this standard, was obtained from a single horse. The serum was reduced to dryness and ground to an impalpable powder. The powder so obtained is preserved in many vacuum tubes under the influence of pentaphosphoric acid. These tubes are kept in absolute darkness at a constant temperature of 5° C. Every two months, or oftener, one of these tubes is opened, dissolved in a solution of glycerin 66 parts and isotonic salt solution 34 parts, and tested.

While the tetanus antitoxin preserved in dry powdered form under the conditions above named is stable, duplicates of the antitoxins made from time to time so as to guard against loss or change will insure the permanence of the standard which has now been established.

METHODS.

In order to obtain reliable and comparable results, it is necessary to take into account all the factors concerned—the composition of the poisons, their concentration, the diluting fluid, length of time the mixtures are allowed to stand, the site of inoculation, etc. All the known factors have been considered in our method of standardizing tetanus antitoxin, so that it is our belief that we now have as accurate and satisfactory a standard for this antitoxin as is the case with diphtheria.

Careful regard must be had for the following points in order to obtain uniform results:

The diluting fluid.—Salt solution containing 0.85 per cent chemically pure sodium chlorid sterilized by boiling is used for diluting both the toxine and the antitoxin.

Time and temperature.—The mixtures of toxine and antitoxin are kept one hour at room temperature in diffused light before injection into the guinea pig.

Amount.—A total of 4 c. c. of the toxine-antitoxin mixture is injected into the guinea pig. If the toxine-antitoxin mixture does not equal 4 c. c., salt solution equal in amount to the difference is used to wash out the syringe, so that the total amount injected and the pressure effects are always equal.

Concentration of the toxine.—One-tenth of a gram of the dried tetanus toxine (A) is dissolved in 166.66 c. c. salt solution. One c. c. of this dilution equals the test dose (L + dose).

Concentration of the antitoxin.—The dilution of the antitoxic serum should follow the tables on page 50.

Guinea pigs. The guinea pigs should weigh 350 grams. Animals weighing from 300 to 400 grams are allowable in preliminary rough testing.

Site of injection.—The injection is always given subcutaneously into the tissues of the abdomen about the level of the umbilicus.[a]

Time of death.—The number of immunity units contained in the serum is determined from the amount given the animals that are living ninety-six hours after the inoculation of the mixtures.

An example of a test.—Carefully tare a weighing bottle, then add approximately 20 to 50 mg. of the dried poison. Again carefully weigh. Dissolve the toxine in the weighing bottle with salt solution (0.85) in the proportion of 0.1 gram of the dried poison to 166.66 c. c. of the salt solution. This proportion is used for the reason that each cubic centimeter of this solution will represent 0.000,6 gm. of the original dried poison (= 100 MLD's). This proportion is taken because it is very convenient in measuring out the test dose, which represents 1 c. c. of the solution. Thus:

---

[a] It is very important to inject the animals in the same place on account of the relation to the motor nerve endings and the distance to travel to the central nervous system.

44.5692 gm., bottle + toxine.
44.5300 gm., bottle.

.0392 gm., toxine.
0.1 gm. : 166.66 c. c. :: 0.0392 : x.
x = 65.33 c. c.

In other words, if the quantity of toxine placed in the weighing bottle should weigh, as in this instance, just 0.0392 gm., carefully deliver from an accurately graduated burette just 65.33 c. c. salt solution into the weighing bottle; and, as before stated, each cubic centimeter of this solution will be the L + or test dose.

Now dissolve the serum of unknown value in accordance with the table of dilutions (page 50) and mix aliquot parts of the serum with the test dose of toxine, as follows:

TABLE No. 1.

No. of guinea pig.	Weight of guinea pig (grams).	Subcutaneous injection of a mixture of		Time of death.
		Toxine (test dose).	Antitoxin.	
		Gram.	*c. c.*	
1.......	360	0.0006	0.001	2 days, 4 hours.
2.......	350	.0006	.0015	4 days, 1 hour.
3.......	350	.0006	.002	Symptoms.
4.......	360	.0006	.0025	Slight symptoms.
5.......	350	.0006	.003	No symptoms.

According to this series the guinea pig which received 0.0015 c. c. of the serum died in four days and one hour. Therefore 0.0015 c. c. of the serum contains one-tenth of an immunity unit, as the unit has been defined as ten times the least amount of antitetanic serum necessary to save the life of a 350-gram guinea pig 96 hours against the official test dose. This serum would, therefore, contain just 66 units per cubic centimeter.

On account of the importance of the L+ or test dose the following table is given showing the results of these tests with Toxine A.

TABLE No. 2.—*Showing the results of the test (L+) dose of tetanus toxine A from January 17, 1907, to April 7, 1908.*

Guinea pig No.	Weight in grams.	Date.	Amount of toxine A.	Amount of antitoxin.	Time of death.	
			Gram.		*Days.*	*Hours.*
306..	290	Jan. 17, 1907	0.0006	1 unit.....	4	0
326..	300	Jan. 24, 1907	.0006	1 unit.....	3	3
334..	315	....do.......	.0006	1 unit.....	3	7
387..	350	Feb. 20, 1907	.0006	1 unit.....	4	19
388..	350	....do.......	.0006	1 unit.....	4	8
389..	350	..do.....	.0006	1 unit..	4	16

TABLE No. 2.—*Showing the results of the test (L+) dose of tetanus toxine A from January 17, 1907, to April 7, 1908*—Continued.

Guinea pig No.	Weight in grams.	Date.	Amount of toxine A.	Amount of antitoxin.	Time of death.	
			Gram.		*Days.*	*Hours.*
400	330	Feb. 25, 1907	0.0006	1 unit	4	21
401	315	do	.0006	1 unit	5	7
407	335	do	.0006	1 unit	4	16
408	315	do	.0006	1 unit	4	9
415	300	Mar. 5, 1907	.0006	1 unit	3	0
416	325	do	.0006	1 unit	3	10
422	300	do	.0006	1 unit	3	9
423	325	do	.0006	1 unit	3	10
430	290	Mar. 22, 1907	.0006	1 unit	4	11
431	290	do	.0006	1 unit	4	14
454	300	Mar. 28, 1907	.0006	1 unit	4	1
455	300	do	.0006	1 unit	3	18
509	300	Apr. 13, 1907	.0006	1 unit	3	17
510	295	do	.0006	1 unit	3	17
524	280	May 3, 1907	.0006	1 unit	3	18
525	290	do	.0006	1 unit	3	11
538	300	May 17, 1907	.0006	1 unit	3	20
539	320	do	.0006	1 unit	3	7
556	330	May 24, 1907	.0006	1 unit	2	20
557	350	do	.0006	1 unit	3	10
615	375	July 28, 1907	.0006	1 unit	2	22
616	380	do	.0006	1 unit	3	10
625	350	July 31, 1907	.0006	1 unit	3	13
626	350	do	.0006	1 unit	4	12
639	350	Sept. 6, 1907	.0006	1 unit	3	4
640	350	do	.0006	1 unit	3	2
654	325	Sept. 24, 1907	.0006	1 unit	5	17
655	370	do	.0006	1 unit	6	19
679	325	Nov. 11, 1907	.0006	1 unit	3	19
680	350	do	.0006	1 unit	4	0
683	350	Dec. 7, 1907	.0006	1 unit	3	15
684	360	do	.0006	1 unit	3	13
702	370	Dec. 19, 1907	.0006	1 unit	3	23
757	350	Apr. 7, 1908	.0006	1 unit	4	1
758	350	do	.0006	1 unit	3	18
759	350	do	.0006	1 unit	4	0

NECESSITY FOR THIS STANDARD.

We now know that when the symptoms of tetanus have developed the toxine has combined with the motor nerve cells and that the union between the cell and the poison is too strong for the antitoxin to break up. However, if the antitoxin is given in time the toxine is neutralized and rendered harmless. Tetanus antitoxin is therefore an exceedingly valuable prophylactic and for this purpose is coming into more general use both in human and veterinary practice.

Whether used as a prophylactic or curative agent it is important to use a potent serum. The law of July 1, 1902,[a] not only insures serums of useful potency, but also establishes uniformity among the producers of tetanus antitoxin in America. The necessity of establishing uniformity for this product in American markets is evident from the following table:

Table No. 3.—*Showing the differences in the methods of testing tetanus antitoxin before the adoption of the American unit.*

Name.	Labeled.	Contents (cubic centimeters).	Units claimed according to special methods of standardization, per cubic centimeter.	Units according to the American standard, per cubic centimeter.
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).	One immunizing dose (No. 2929F).		0. 5	434
New York City department of health (submitted by Dr. W. H. Park for comparison).			700	166
H. K. Mulford Co. (bought on open market).	1,000,000 immunizing units (No. 2121).	10	100,000	77
Farbenfabrik vorm. Meister Lucius & Bruning, Hoechst, a/M (submitted for comparative tests).	Tetanus Antitoxin, 5 fach. Normal, Prüfungs dose = 1/500.			333
H. K. Mulford Co., No. 9971 (submitted for tests).	60,000,000 units	10	6,000,000	90
Parke, Davis & Co			60,000	700
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).			.75	769
Serum Antitetanique, Pasteur Institute, 10 c. c. fluid.	Unit value not stated.	10	Not stated.	40
Serum Antitetanique, Pasteur Institute	Unit value not stated.	10	Not stated.	40
Tizzoni, Antitossina del Tetano, No. 2912, Dry.	Unit value not stated.		Not stated.	[b] 83. 3
Pasteur Institute, Serum antitetanique, II-7.	Unit value not stated.	10	Not stated.	66
Institute Bacteriologique de Lyon et du Sud-Est.	Unit value not stated.	10	Not stated.	([c])

[a] Dissolved in 26. c. c.

[b] This equals 833 units per gram of the dry serum.

[c] Less than 50.

Tetanus antitoxin is made by the following laboratories in the United States:

Parke, Davis & Co., Detroit, Mich. (license No. 1); H. K. Mulford Company, Philadelphia, Pa. (license No. 2); Lederle Antitoxin

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[a] An act regulating the sale of viruses, serums, toxins, and analogous products, etc. Approved July 1, 1902.

Laboratories, New York, N. Y. (license No. 17); New York State department of health, Albany, N. Y.; and the department of health of the city of New York, N. Y.

HISTORICAL REVIEW.

Compared with the major plagues of man lockjaw has always been a rare disease. It is on account of the peculiar and characteristic spasms that it early attracted attention. In the writings of Hippocrates cases of tetanus are described, diagnosed, and prognosis given. From the learned Aretaeus of Kappadoza we have a description of tetanus that holds until modern times.[a] Aretaeus described an opisthotonos, an emphrothotonos, and a tetanus, depending upon whether the muscles of the back, the abdomen or the general muscular system were affected. To this classification pleurothotonos was later added when the muscles of one side were especially affected.

Throughout the Middle Ages our knowledge of tetanus remained at a standstill. The symptoms of the disease so plainly indicated that the lesions are localized in the nervous system that, with the introduction of studies in pathologic anatomy, the brain, spinal cord, and nerves were studied to determine the seat and nature of the illness. It was not, however, until about the end of "the sixties" in the last century that experimental studies in wound infections began to throw light upon the chaos of theories. A review of the historical development of these theories is exceedingly interesting, for they mirror the prevailing thought upon the nature of disease, as it developed from that of evil spirits, through the "humoral" theory, the realm of miasm and noxious effluvia, to the germ theory.

It was long known that tetanus was a complication of wounds. Micheles, 1797, thought it was due to irritation of the peripheral nerves from foul secretions in certain wounds ("wound insults"). Tetanus could not escape the rheumatism theory, which has been such an alluring catch-all for symptoms and diseases difficult of explanation. In cases of wound tetanus in which no plausible explanation seemed possible it was called rheumatic wound tetanus. "Taking cold" was assigned its usual rôle here as elsewhere. When no assignable cause seemed at hand the disease was given the learned title "idiopathic tetanus."

About 1860 Heiberg and Rose, and Billroth and Spencer Wells[b] attached tetanus to the list of zymotic diseases and believed it due to a miasm, the spasms being caused by a poison in the blood similar to strychnine.

---

[a] A part of the historical data are taken from Von Lingelsheim's article on Tetanus in Kolle & Wassermann's Handbuch der pathogenen Mikroorganismen, vol. 2, p. 566–600.

[b] Billroth and Spencer Wells. Wiener med. Presse, 1869, p. 36.

E. Rose[a] 1870, described a particular form of tetanus following wounds in the area of the twelfth cerebral nerve which he called "head tetanus" or "tetanus hydrophobicus."

An important milestone in the history of tetanus was passed when Strümpell,[b] 1876, declared tetanus to be an infection. The first experiments to prove this view failed.

Arloing and Tripier[c] did not succeed in transferring the disease to dogs and rabbits by carrying over the blood and the pus from tetanus wounds.

Billroth[d] and Schultz[e] also obtained negative results with dogs.

Carle and Rattone,[f] 1884, were the first to obtain positive results in demonstrating the transmissibility of the disease. They injected a number of rabbits with the washings from the neighborhood of an acne pustule from which the tetanus originated. The rabbits were injected either into the sciatic nerve or into the muscles of the back. After an incubation period of two to three days 11 of the 12 rabbits showed typical tetanic spasms. They also succeeded in carrying the infection from animal to animal.

By injecting earth taken from different places into 140 mice, rabbits, guinea pigs, etc., in studying the microorganisms of soil (ground tetanus), Nicolaier,[g] 1884, produced symptoms of tetanus in 69 of them and always found a slender rod in the pus. Therefore he concluded that there exists a bacillus that causes tetanus in deep wounds of mice, rabbits, and guinea pigs. In cultures Nicolaier was unable to separate this bacillus from the other microorganisms with which it was associated, but inoculation experiments with mixed cultures produced tetanus.

The next year (1885) Nicolaier,[h] working under Flügge in the Göttingen Hygienic Institute, made noteworthy advances upon the subject. He showed that each injection into mice, guinea pigs, and rabbits caused a disease with tetanic spasms, while dogs were refractory. Nicolaier saw in the pus of the wounds this slender bacillus, which he considered the cause of the infection, but again was unable to obtain it in pure culture, although he carried on a mixed culture upon coagulated calf's blood serum.

---

[a] Nicolaier, Arthur: Zur Aetiologie des Kopftetanus (Rose). Virch. Arch., vol. 128, 1892, p. 1–19.

[b] Strümpell: Ueber die Ursachen der Erkrankungen des Nervensystems. Arch. f. klin. Med., vol. 35, p. 14–15.

[c] Arloing and Tripier: Gaz. med. de Paris, 1870, p. 337.

[d] Billroth: Allgemeine chirurgische Pathologie und Therapie. Berlin, 1882, p. 504.

[e] Schultz: Ueber einer Kumulation von Tetanusfällen im Stadtkrankenhause zu Rostock. Rostock, 1876, p. 13.

[f] Carle and Rattone: Studio sull' enziologia del tetano (communicazione preventiva). Giorn. dell' R. Accad. di med. di Torino, Marzo, 1884.

[g] Nicolaier, Arthur: Ueber infectiosen Tetanus. Deut. med. Woch., vol. 10, 1884, p. 842–844.

[h] Nicolaier, Arthur: Beiträge zur Aetiologie des Wundstarrkrampfes. Inaug. disserta., Göttingen, 1885.

Soon afterwards Rosenbach,[a] 1886, found a similar bacillus with a round terminal spore in a case complicating frost gangrene in man and transferred the infection to animals in the pus.

In 1889, with the aid of anaerobic technic, Kitasato[b] for the first time grew the bacillus in pure culture and by successful inoculation experiments finally proved that this bacillus was the real cause of tetanus. Kitasato succeeded in isolating the tetanus bacillus, first described by Nicolaier and later by Rosenbach, not alone by understanding anaerobic methods but by taking advantage of its resisting spore. This bacillus appears in the pus of wounds of man and animals having tetanus. It often forms spores in the pus, but if the pus is examined early it may contain spore-free rods. He planted the material containing the bacillus upon agar slants, which were incubated one to two days. The mixed cultures so obtained were heated one hour at 80° C. and then transplanted to agar plates. Kitasato designed for this purpose a special shaped apparatus in which hydrogen was used to replace the air.

Kitasato[c] further showed that the tetanus bacillus is not found in the heart's blood of mice dead of tetanus and therefore concluded that we are dealing with an intoxication and not with an infection.

It was in the following year, 1890, that Behring and Kitasato[d] published their epoch-making work upon the tetanus toxin and tetanus antitoxin, laying the foundation of serum therapy. On account of the great historical and practical importance of this document a transcription of its principal features is given:

> The immunity of rabbits and mice which have been immunized against tetanus depends upon the power of the cell-free serum to render the toxic substances which are produced by the tetanus bacillus harmless.
>
> Experiments show:
>
> 1. The blood of tetanus immune rabbits possesses the property of destroying the tetanus poison.
>
> 2. This property is destroyed by the extravascular blood and the cell-free serum obtained from it.
>
> 3. This property is of such a stable nature that it is also effective in the bodies of other animals, so that we are in a position, by means of the transfer of blood serum, to accomplish noteworthy therapeutic results.

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[a] Rosenbach: Zur Aetiologie des Wundstarrkrampfes beim Menschen. Arch. f. klin. Chir., Berl., vol. 34, p. 306–317.

[b] Kitasato, S.: Ueber den Tetanusbacillen. Zeit. f. Hyg., vol. 7, 1889, p. 225.

[c] Kitasato, S.: Experimentelle Untersuchungen über das Tetanusgift. Zeit. f. Hyg., vol. 10, 1891, p. 304.

[d] Behring, E., and Kitasato, S.: Ueber das Zustandekommen der Diphtherie-Immunitat und der Tetanus-Immunitat bei Theiren. Deut. med. Woch., vol. 16, 1890, p. 1113–1114.

4. The property of destroying the tetanus poison is absent in the blood of animals which have not been immunized against tetanus. If the tetanus poison be given to susceptible animals it may be demonstrated in the blood and other body fluids after the death of the animal.

For instance: A rabbit was immunized against tetanus to such a high degree of immunity that it was able to withstand 10 c. c. of a virulent culture containing the tetanus organism, 0.5 c. c. of which was sufficient to kill a normal rabbit. This rabbit was not only able to withstand the infection of living tetanus bacilli, but was also immune to the tetanus poison. Thus it withstood without any symptoms twenty times the quantity of tetanus poison sufficient to kill a normal rabbit.

This rabbit was bled from the carotid, and before the blood clotted 0.2 c. c. was given to a mouse and 0.5 c. c. to another. After twenty-four hours both of these treated mice and two control mice were inoculated with a virulent tetanus bacillus. The amount injected was so great that the control animals showed tetanus in twenty hours and were dead in thirty-six hours. Both treated mice, on the other hand, remained well.

The larger quantity of blood was allowed to stand until the serum separated. Of this serum six mice were injected with 0.2 c. c. each into the peritoneal cavity. Twenty-four hours later they were infected and remained well, while the control mice died in less than forty-eight hours.

It was further found that the serum had a therapeutic value in that the mice were first infected and afterwards protected by injecting the serum into the peritoneal cavity.

They further made experiments to demonstrate that the serum has an enormous power of destroying the poison.

Of a tetanus culture ten days old which was freed from bacilli by filtration, 0.00005 c. c. was sufficient to kill a mouse in four to six days and 0.0001 c. c. was sufficient to kill a mouse with certainty in two days. However, 5 c. c. of the serum from the tetanus immune rabbit was mixed with 1 c. c. of this culture, and the serum was allowed to act twenty-four hours upon the culture containing the tetanus poison. Of this mixture, four mice were given each 0.2 c. c., containing 0.0033 c. c. of the culture, or more than 300 times the dose otherwise fatal for mice. All four of the mice remained well. The control mice, on the other hand, died in thirty-six hours with 0.0001 c. c. of the fluid. The mice which were treated as above described, as well as those which had received the mixture of tetanus poison with serum, so far as can be told, remained well. Later they were injected repeatedly with virulent tetanus bacilli, which they withstood without a trace of symptoms.

This fact is of very particular note, because in the innumerable experiments no mouse, no rabbit, in fact no animal so far tested has shown a natural immunity against tetanus. We therefore may draw the conclusion that the above-mentioned explanation of the conditions of immunity which may be obtained promptly and without difficulty, and which may be accomplished without harm to the animals in the process of immunization, will prove of far-reaching value. It is to be understood that control animals were tested with the blood and serum of nonimmune rabbits. The same may be said to be true of cattle and sheep.

We permit ourselves from our results to venture the conclusion that, after finding a therapeutically potent substance useful for animals, it will also be useful in the treatment of diphtheria and tetanus in man. We only desire to call attention to one thing in conclusion: In former times the transfusion of blood was considered to be a heroic, but in certain cases a very powerful, curative agent. In later times it was thought that physiological salt solution was able to accomplish the same result. On the other hand, our experience makes memorial the phrase "Blut ist ein ganz besonderer saft."

THE STABILITY OF TETANUS TOXINE.

From a practical standpoint it is important to obtain a stable poison. Herein lies the crux of the problem so far as the standardization of tetanus antitoxin is concerned. Tetanus toxine in solution can not be depended upon. Its strength varies so, that if the soluble poisons are used to measure the value of the antitoxic serums, as is the case with the German method, it is found necessary to redetermine by a series of mice the strength of the toxine each time a serum is tested. Diphtheria toxine in solution, after a period of ripening, comes to a stage of equilibrium and after this period is reached retains its toxicity for a long time. This fact is of signal service in the standardization of diphtheria antitoxin. The fact that tetanus toxine does not exhibit the same constancy in solution has thrown much confusion and no little difficulty into the work of standardizing its antitoxin.

Our first efforts therefore were to obtain a stable poison. In this we fortunately succeeded by precipitating the filtered bouillon culture with ammonium sulphate, drying the precipitate over sulphuric acid, grinding to an impalpable powder, and preserving in vacuo under the influence of pentaphosphoric acid—a very strong dehydrating substance—in a cold, dark place. Under these conditions we have found the dried poison maintains its strength without appreciable alteration at least 25 months, as will be seen by the following table:

TABLE NO. 4.—*The stability of the standard tetanus toxine A.*

Guinea pig No.	Weight in grams.	Date.	Amount toxine subcutaneously.	Time of death.	
			Gm.	*Days.*	*Hours.*
18	355	Mar. 8, 1906	0.000,006	3	0
48	360	Mar. 27, 1906	.000,006	3	11
49	350	do	.000,006	3	3
63	340	Mar. 31, 1906	.000,006	3	10
64	340	do	.000,006	3	16
75	325	Apr. 4, 1906	.000,006	2	12
76	325	do	.000,006	3	5
89	350	Apr. 7, 1906	.000,006	3	11
151	335	June 12, 1906	.000,006	4	12
152	340	do	.000,006	6	9
153	340	do	.000,006	6	9
168	310	June 26, 1906	.000,006	3	8
199	330	July 26, 1906	.000,006	5	9
206	325	Sept. 10, 1906	.000,006	3	6
227	350	Nov. 28, 1906	.000,006	4	11
228	350	do	.000,006	4	0
239	360	Dec. 3, 1906	.000,006	3	14
240	365	do	.000,006	4	4
245	335	Dec. 5, 1906	.000,006	4	0
246	335	do	.000,006	7	23
251	395	Jan. 3, 1907	.000,006	3	4
252	395	do	.000,006	3	12

TABLE NO. 4.—*The stability of the standard tetanus toxine A*—Continued.

Guinea pig No.	Weight in grams.	Date.	Amount toxine subcutaneously.	Time of death.	
			Gm.	*Days.*	*Hours.*
257	305	Jan. 5, 1907	0.000,006	2	14
258	315	do	.000,006	2	13
263	300	Jan. 7, 1907	.000,006	3	20
264	305	do	.000,006	4	8
395	395	Feb. 20, 1907	.000,006	5	0
404	315	Feb. 25, 1907	.000,006	3	7
411	340	do	.000,006	4	4
412	320	do	.000,006	3	17
434	300	Mar. 22, 1907	.000,006	4	17
435	300	do	.000,006	3	18
458	300	Mar. 28, 1907	.000,006	4	19
459	300	do	.000,006	4	18
552	320	May 17, 1907	.000,006	4	19
553	330	do	.000,066	4	10
593	340	June 25, 1907	.000,006	5	14
604	350	July 12, 1907	.000,006	3	4
609	300	July 20, 1907	.000,006	4	8
610	310	do	.000,006	5	10
629	370	Aug. 29, 1907	.000,006	4	0
630	390	do	.000,006	4	8
643	325	Sept. 10, 1907	.000,006	5	15
644	330	do	.000,006	5	8
658	340	Oct. 11, 1907	.000,006	4	0
659	350	do	.000,006	4	4
663	350	do	.000,006	4	0
664	355	do	.000,006	3	12
703	385	Dec. 19, 1907	.000,006	4	20
704	390	do	.000,006	5	20
729	330	Feb. 10, 1908	.000,006	4	15
730	340	do	.000,006	3	19
731	340	do	.000,006	4	9
749	330	Apr. 7, 1908	.000,006	4	0
750	335	do	.000,006	3	17
751	335	do	.000,006	4	2
752	340	do	.000,006	3	23
753	340	do	.000,006	3	23

It has fortunately developed that this dried poison not only retains its virulence when kept under the exact conditions above mentioned, but loses its toxicity rather slowly when exposed to light, heat, and other influences. One of the sealed tubes sent from Washington to Manila by mail arrived there without appreciable loss of toxicity.

This dried poison, which is distributed to manufacturers and other laboratories engaged in the work of standardizing tetanus antitoxin, is therefore well suited to withstand transportation. We recommend, however, that it always be kept in a vacuum desiccator in a dark, cold place.

The effect of sunlight upon this toxine will be seen in the following tables:

THE STABILITY OF THE STANDARD TETANUS TOXINE.

In the following experiments the toxine (No. A.) was placed in small wide-mouthed vials, loosely stopped with a pledget of absorbent cotton and exposed under the conditions stated in each table. Table No. 4, page 16, shows the stability of this toxine when kept under special conditions and serves as a control for tables 5 to 8.

TABLE NO. 5.—*Influence of sunlight.*

[M.L.D = .000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
156.....	375	June 23, 1906	6	18	0.000,004	Exposed 54 minutes to direct sunlight and 60 hours to bright light in front of window.
157.....	385	.....do.......	5	19	.000,005	
158.....	400	.....do.......	4	9	.000,006	
159.....	440	.....do.......	4	6	.000,007	
160.....	480	.....do.......	6	8	.000,008	
171.....	320	June 26, 1906	4	2	.000,006	Exposed 54 minutes, again 66 minutes to direct sunlight; and 5½ days bright light in front of window.
172.....	320	.....do.......	4	0	.000,007	
173.....	320	.....do.......	2	20	.000,008	
193.....	310	July 3, 1906	(a)		.000,006	Same toxine exposed 4 times, about 1 hour each, bright sunlight and in front of window, 13 days.
194.....	350	.....do.......	(a)		.000,007,2	
195.....	370	.....do.......	(a)		.000,008,1	
190.....	370	.....do.......	8	21	.000,006	Control. Same toxine kept in vacuum desiccator in dark at 15° C.
191.....	375	.....do.......	6	20	.000,007,2	
192.....	375	.....do.......	5	23	.000,008,1	

[a] Symptoms.

It is evident from the above that two exposures of one hour each to bright sunlight, and five and one-half days to bright light before a window with a southern outlook had no appreciable effect on the dried toxine. However, four exposures of one hour each upon different days to sunlight and thirteen days in bright light slightly diminished the virulence of the poison.

TABLE NO. 6.—*Influence of temperature 37° C.*

[M.L.D = 0.000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
161.....	375	June 23, 1906	5	20	0.000,004	60 hours at 37° C.
162.....	410	.....do.....	6	1	.000,005	Do.
163.....	480	.....do.....	5	15	.000,006	Do.
164.....	480	.....do.....	6	15	.000,007	Do.
165.....	490	.....do.......	6	6	.000,008	Do.
174.....	320	June 26, 1906	5	1	.000,006	5½ days at 37° C
175.....	320	.....do.......	5	1	.000,007	Do.
176.....	375	.....do.......	4	4	.000,008	Do.

TABLE NO. 6.—*Influence of temperature* 37° *C.*—Continued.

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
196.....	300	July 3, 1906	(a)		0.000,006	13 days at 37° C.
197.....	345	.....do.......	(a)		.000,007	Do.
198.....	360	.....do.......	(a)		.000,008,1	Do.
217.....	300	Sept. 25, 1906	(b)		.000,006	97 days at 37° C.
218.....	325	.....do.......	(b)		.000,007,2	Do.
219.....	330	.....do.......	(b)		.000,009	Do.
220.....	340	.....do.......	(b)		.000,009,9	Do.
221c....	340	.....do.......	2	14	.000,012	Do.c
222.....	350	.....do.......	(b)		.000,012,9	Do.
223.....	360	.....do.......	(b)		.000,014,1	Do.
224.....	390	.....do.......	(d)		.000,015	Do.
225.....	350	Nov. 28, 1906	3	14	.000,004,2	Control; toxine kept under vacuum at 15° C.
226.....	350	.....do.......	4	4	.000,005,1	Do.
227.....	350	.....do.......	4	11	.000,006	Do.
228.....	350	.....do.......	4	0	.000,006	Do.
229.....	350	.....do.......	3	16	.000,007,2	Do.
230.....	350	.....do.......	3	19	.000,008,1	Do.

a Symptoms.
b Slight symptoms.
c No tetanic symptoms; died of pneumonia.
d Marked symptoms.

An exposure of five and one-half days at 37° C. in the incubator had no apparent effect upon the toxine. After ninety-seven days under the same conditions the poison had lost some of its toxicity.

TABLE NO. 7.—*Influence of room temperature, diffuse light, etc.*

[MLD=.000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
231.....	....	Nov. 30, 1906	(a)		0.000,005	163 days in open vial, dark closet, room temperature (June 20 to Nov. 30, 1906).
232.....	....	.....do.......	11	13	.000,006	
233.....	....	.....do.......	6	18	.000,007	
234.....	....	.....do.......	6	14	.000,008	
235.....	....	.....do.......	6	15	.000,009	
236.....	....	.....do.......	6	23	.000,01	
577.....	320	June 18, 1907	(b)		.000,01	363 days in open vial, dark closet, room temperature (June 20, 1906, to June 18, 1907).
578.....	370	.....do.......	(c)		.000,02	
579.....	370	.....do.......	(c)		.000,04	
580.....	375	.....do.......	5	12	.000,06	
581.....	385	.....do.......	4	2	.000,08	
582.....	400	.....do.......	3	21	.000,1	

a Slight symptoms.
b No symptoms.
c Symptoms.

The standard toxine under these conditions has shown marked stability. After a sojourn of one year (363 days) in a dark closet, at room temperature, it lost in virulence. It will be recalled that the poison was kept in an open vial, loosely stoppered with absorbent cotton, so that it had free access to the oxygen and moisture of the air.

TABLE No. 8.—*Influence of diffuse light, etc.*

[M.L.D.=0.000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
204	360	July 26, 1906	[a]		0.000,008.1	36 days at room temperature in diffuse light.
205	375	do	4	17	.000,009	
206	375	do	5	17	.000,009.9	
207	375	do	6	3	.000,011.1	

[a] Symptoms.

From this single experiment it will be seen that the toxine exposed to diffuse light at room temperature diminished slightly in toxicity after thirty-six days of such exposure.

It is therefore evident that the dried poison, which we use for the purpose of standardizing tetanus antitoxin, has sufficient stability for practical purposes.

The work of other investigators upon the stability of tetanus toxine and the influence of chemical agents are briefly abstracted in the following:

The first exact work upon the stability of tetanus toxine was done by Kitasato[a] in 1891. This pioneer paper contains an extensive series of tests upon the poison, showing the influence of physical agents and a long list of chemical substances. Kitasato found that the filtrate of tetanus cultures is very sensitive to heat; 65° C. and above is sufficient to destroy it within a few minutes (5 minutes and less). It will sometimes withstand 60° C. for fifteen minutes, but is destroyed at 60° C. for twenty minutes.

It retained its toxic properties when heated an hour and a quarter at 55° C., but lost its power after one and a half hours.

The filtrate of tetanus culture may be dried and retain its toxicity, but this depends upon the manner in which the drying is carried out. In the dessicator over sulphuric acid the filtrate does not lose its properties. The same may be said if it is dried in the air at room temperature, although it is somewhat weakened.

On the other hand, the filtrate was totally destroyed if it is dried in the incubator. The filtrate gradually loses its strength at a temperature of 35° to 37° C.

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[a] Kitasato, S.: Experimentelle Untersuchungen über das Tetanusgift. Zeit. f. Hyg., vol. 10, 1891, p. 267–305.

The filtrate placed in the window in diffuse light gradually lost its properties. It lasted, however, a long time before it was entirely destroyed. After it had been nine or ten weeks at the window it was still toxic, but in large doses. The filtrate kept cold in a dark room retained its toxic properties much longer, for after three hundred days it was still equally as toxic as when fresh.

Direct sunlight totally destroys the tetanus poison in from fifteen to eighteen hours.

Diluting the poison with water or bouillon does not seem to harm it in any way.

Hydrochloric acid influences the tetanus poison when added in the proportion of 0.55 per cent within one hour, and in the amount of 0.1365 per cent within twenty-four hours completely destroys the poison.

From similar experiments with many chemical substances Kitasato drew the conclusion that the tetanus poison is very susceptible to acids, especially mineral acids, as well as alkalies.

Tizzoni and Cattani,[a] 1891, have shown that silver nitrate, sublimate, and hydriodic acid alter the toxicity of filtered tetanus cultures; carbolic acid, chlorine water, and iodidum trichloride were also found to have the same effect. Tizzoni and Cattani had much difficulty at first in immunizing animals with large doses of the tetanus toxine in order to obtain potent antitoxin. On account of the virulence of the poison many animals were sacrificed. They found that treating the toxine with iodine trichloride was useful in order to weaken the poison, and thus permit the use of large doses, which could then be rapidly increased.

Vaillard and Vincent,[b] 1891, showed that the tetanus toxine is modified or destroyed by temperatures exceeding 65° C. It is considerably attenuated when heated in a closed flask for forty minutes at 60° C. or twenty minutes at 62° C. Heating for thirty minutes at 65° C. in closed flasks renders it inactive. Kept in a closed flask free from air and light the liquid toxine keeps its activity for a long time; after four months there is no change in its toxicity. Exposed to the air in a thin layer it loses its activity after a month; this action is more rapid and greater in sunlight.

Acidification of the liquid by tartaric acid does not modify its toxicity. It can be dried in a vacuum over H_2SO_4 and is extremely toxic.

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[a] Tizzoni, G., and Cattani, Giuseppina: Ueber die Eigenschaften des Tetanus-Antitoxins. Centblt. f. Bakt., Ref., vol. 9, 1891, pp. 189, 685.

[b] Vaillard, L., & Vincent, H.: Contribution a l'étude du tétanos. Ann. Inst. Pasteur, vol. 5, 1891, p. 1.

Tetanus toxine is insoluble in alcohol. It is precipitated by phosphate of lime or of aluminum; retains its toxicity for a long time; it resists well in this state the action of the air.

Vaillard and Rouget[a] proved that the heating at 65° to 67° C. for five, ten, or fifteen minutes diminishes considerably but does not destroy the toxine. When heated ten or fifteen minutes to 70° or 75° C. and one hour at 80° C. if injected in very large amounts is toxic for guinea pigs. They showed that the spores contained a certain amount of toxine which resisted 80° C. for one hour.

Martin[b] also showed that the toxine is very sensitive to heat; 65° C. or above for five minutes completely destroys it; but it withstands 60° C. for fifteen minutes and 55° C. for an hour and a quarter; much longer at lower temperatures. He dried it over H_2SO_4 without loss of toxicity. If dried in the incubator it loses its toxicity. It was destroyed in nine or ten weeks by diffused daylight; fifteen to eighteen hours direct sunlight completely destroys it.

Lumière and Chevrottier[c] found that solutions of permanganate of potash and sulphate of iron, and the salts of cerium, cobalt, and nickel markedly attenuate the toxine of tetanus.

Fermi and Pernossi[d] draw the following conclusions from their studies of the tetanus poison:

Agar cultures are the most poisonous. Next come those on gelatin, and lastly those in bouillon. Chickens, snakes, turtles, and tritons are immune to the poison. In the above-mentioned animals this toxine may remain and retain its virulence for three days, and even longer. Filtrates from agar and gelatin cultures are more resistant to heat than those from bouillon. Like the enzymes, the purer the tetanus poison the less stability does it possess. Dissolved in water, the tetanus poison is rendered inert by a temperature of 55° C., but in the dry state it can be heated to 120° C. without loss of virulence. When the dry poison is mixed with ether or chloroform and heated to 80° C. it is destroyed; but with amylic alcohol or benzol a temperature of 100° is required to accomplish this result. Dissolved in water this poison is destroyed by direct sunlight after

[a] Vaillard, L., & Rouget, J.: Contribution a l'étude du tétanos. Ann. Inst. Pasteur, vol. 6, 1892, p. 385.

[b] Martin, S.: Further report on the chemical pathology of tetanus. Report of med. officer, Local Govt. Board, London, 1894-5, vol. 24, 1896, pp. 505-513.

[c] Lumière, A. & L., & Chevrottier, J.: Action des oxydases artificielles sur la toxine tétanique. Compt. rend. acad. sci., vol. 138, 1904, pp. 652-654.

[d] Fermi, Cl., and Pernossi, Leone: Ueber das Tetanusgift. Vergleichende Studien mit Berücksichtigung anderer, Gifte und der Enzyme. Centblt. f. Bakt., 1 Abt., vol. 15, 1894, pp. 303-310. Ref., Vaughan & Novy's "Cellular toxins," 1902, p. 62-63.

an exposure of eight to ten hours (with the highest temperature on a blackened thermometer at 56° C.) and after fifteen hours when the temperature does not exceed 37° C. In the dry state the tetanus poison can be exposed to the direct sunlight for one hundred hours without loss of virulence. Under the action of an electric current of 0.5 ampere, continued for two hours, the substance becomes inert. Gastric juice destroys the poison through the activity of the hydrochloric acid and not by virtue of the pepsin. Ptyalin, diastase, and emulsin have no action on it. The effect of trypsin has not been satisfactorily determined. Putrefactive germs do not destroy the poison. The living, but not the dead, intestines of guinea pigs and cats destroy its toxicity. The poison may be eliminated by the kidneys and retain its properties in the urine; it is not a ferment.

Ritchie [a] states that tetanus toxine under the influence of hydrochloric acid loses with comparative readiness its virulently poisonous properties. It does not, however, so readily lose its capacity of producing immunity, and when all trace of toxicity has disappeared the capacity of producing immunity still remains. The less poisonous substances produced in the modified toxine are probably of the nature of toxoids.

Tetanus toxine is also susceptible to the action of alkalies, such as sodium hydrate and sodium carbonate, under which it loses its toxicity.

Noguchi,[b] 1907, found that eosin, if present in cultures containing tetanus spores, prevents the germination of these spores when its concentration (in glucose bouillon) reaches 0.2 per cent.

The toxine production of tetanus bacilli grown in eosinized culture media diminishes as the concentration of the eosin increases. This effect is brought about partly by the restraining action of the dye on vegetation and partly by its detoxicating action upon the poison.

The toxine-producing power and the virulence of tetanus bacilli are not permanently modified by contact with eosin for a long period or by successive cultivations in eosinized media.

Burckhard[c] found that formalin when added to tetanus toxine in the proportion of 1 to 250 was sufficient to protect a mouse against a dose of tetanus toxine which caused the death of the control in twenty-four hours. Burckhard does not seem to have determined the fatal dose of his tetanus toxine.

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[a] Ritchie, James: Artificial modifications of toxins with special reference to immunity. Journ. hygiene, vol. 1, 1901, pp. 125–144.

[b] Noguchi, Hideyo: The nature of the antitetanic action of eosin. Journ. exper. med., vol. 9, no. 3, May 25, 1907, pp. 281–290.

[c] Burckhard, G.: Zwei Beitrage zur Kenntniss der Formalinwirkung. Centblt. f. Bakt., 1 Abt, vol. 18, 1895, pp. 257–264.

One of us (Anderson),[a] 1907, found that when tetanus toxine is exposed to 5 per cent formalin for six hours a guinea pig is able to withstand 100 MLD of this formalinized toxine. Three per cent formalin after twenty-four hours' exposure protects against the toxine; it destroys a part of the toxine in one hour, its action increasing with the length of exposure.

THE TETANUS TOXINE.

On account of its virulence, its solubility, and the characteristic contractions which it induces the poison of tetanus has been a convenient and favorite subject of investigation. It was the first of the bacterial toxines to give fruitful results in the realm of serum therapy.

Tetanus toxine is readily soluble in the medium in which the culture grows whether fluid or solid. We shall presently see that the filtered culture, called the "tetanus toxine," is really a complex substance, containing various poisons and other bodies.

The particular poison that concerns us especially is the *tetano-spasmine*, which produces the convulsions characteristic of the disease. This poison is a type of a true toxine. It is readily rendered inert by heat, contains both a toxophore and a haptophore group, induces antibodies when introduced into susceptible organisms, and produces symptoms only after a definite period of incubation. Its poisonous action is destroyed by the digestive juices. In all these characteristics tetanus toxine resembles diphtheria toxine. It is one of the most poisonous substances known. As small an amount as 0.000,006 gram of our standard precipitated toxine invariably kills a guinea pig weighing 350 grams. As the precipitate consists mostly of albumins, peptone, amido acids, volatile substances, ammonium sulphate, and other salts it will be seen that but a small proportion of the weight consists of pure poison. Our standard toxine is by no means as strong as other tetanus poisons that have been prepared.

Brieger and Cohn[b] found their strongest tetanus poison killed mice weighing 15 grams when given subcutaneously in doses of 0.000,000,05 gram; smaller doses, such as 0.000,000,01 gram, caused more or less tetanic symptoms in mice. If 0.000,000,05 gram kills a mouse weighing 15 grams Brieger and Cohn calculate that 0.000,23 gram would be a fatal dose for a man weighing 70 kilograms.

Tetanus toxine is not equally toxic for all species of animals. On the other hand, there is an extraordinary constancy in its toxicity upon individuals of the same species. The same quantity of poison per gram weight of a particular animal always produces similar results.

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[a] Anderson, John F.: The antiseptic and germicidal properties of solutions of formaldehyde and its action upon toxines. Hyg. Lab. Bull. No. 39. U. S. Pub. Health and Mar. Hosp. Serv., Wash., 1907, pp. 47. 8°.

[b] Brieger, Ludwig, and Cohn, Georg: Untersuchungen das Tetanusgift. Zeit. f. Hyg., vol. 15, 1893, pp. 1–10.

In 1893 Wladimiroff[a] conducted under Behring's direction a series of experiments to determine the minimal fatal dose for various species of animals. Considering the susceptibility to tetanus toxine for mice as 1, we have the following results. The minimal fatal dose is calculated to the body weight:

Animal species.	Minimal fatal dose.	Susceptibility.
White mice	1 : 500.000	1
White rats	1 : 50,000	0. 1
Guinea pigs	1 :1,000,000	2
Rabbits	Greater than 1 : 24,000.	Considerably less than $\frac{1}{20}$.
Goats	1 :250,000	About $\frac{1}{2}$.

Expressed in figures of minimal lethal doses for the above-mentioned animals we have the following:

Animal species.	Minimal fatal dose.	Minimal fatal dose expressed in minimal fatal dose for mice.
White mice	0.00004	1
White rats	0.004	100
Guinea pigs	0.0002	5
Young goats	0.1	25.000
Medium size rabbits	5.0	125.000

According to Behring, 1 minimal lethal dose per mouse is equivalent to 12 for a horse, 6 for a guinea pig, $\frac{1}{2}$ for a goat, $\frac{1}{150}$ for a rabbit, $\frac{1}{1000}$ for a goose, $\frac{1}{4000}$ for a pigeon, and $\frac{1}{30000}$ for a chicken. That is, the horse is 12 times as susceptible as the mouse, and the mouse is 30,000 times more susceptible than the chicken.

Knorr[b] in his paper on the subject gives a scale of susceptibility to tetanus poison. He finds the horse to be the most susceptible animal. If we take the dose that kills 1 gram of horse as unity, the amounts of poison required by the following animals is as follows:

1 gram of guinea pig	2 times as much
1 gram of goat	4 times as much
1 gram of mouse	13 times as much
1 gram of rabbit	2,000 times as much
1 gram of hen	200,000 times as much

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[a] Wladimiroff, A.: Ueber die Antitoxinerzeugende und immunisirende Wirkung des Tetanusgiftes bei Thieren. Zeit. f. Hyg., vol. 15, 1893, pp. 405–422.

[b] Knorr: "Das Tetanusgift und seine Beziehg. zum tier. Organismus." Münch. med. Woch., 1898, 321, 362.

There is always a period of incubation in the animal organism. The duration of this period varies with the species. As a rule, the period of incubation is shorter the more susceptible the animal and the higher the dose. According to Behring:

	Incubation (hours).
13 lethal doses per gram, mouse	36
110 lethal doses per gram, mouse	24
333 lethal doses per gram, mouse	20
1,300 lethal doses per gram, mouse	14
3,600 lethal doses per gram, mouse	12

The period of incubation is never shorter than eight hours.

We have found in the guinea pig that the period of incubation varies inversely with the size of the dose; that is, within certain limits the larger the dose the shorter the period of incubation. This period may vary from about eighteen hours to about five days.

We have found that there is a direct relation between the period of incubation and the severity of symptoms in guinea pigs. Guinea pigs that show symptoms within forty-eight hours after injections of tetanus toxine (either M. L. D. or L+) invariably die. Guinea pigs that show symptoms on the third day usually die, a very small percentage recovering. The longer the onset of symptoms is delayed, the milder the disease and the greater the chances of recovery. These statements are based upon a tabulation of 600 serial cases.

Tetanus toxine is harmless when given by the mouth. It is not absorbed from the intact intestinal tract and is destroyed by the digestive juices.

As a result of work upon guinea pigs, Ransom[a] concluded that tetanus toxine, even in very large doses, is harmless so long as the intestinal tract is intact; that the poison is not absorbed either from the stomach or from the intestines, and in consequence neither the poison nor its antitoxine appears in the blood; and, finally, that the poison is not destroyed in the intestinal canal, but flows through the entire canal and is thrown off *per anum*.

Carrière, however, and several other investigators were unable to detect any toxine in the excreta after the introduction of large doses of toxine by the mouth.

Carrière[b] accordingly made experiments to determine where tetanus toxine became innocuous. He found that the poison was attacked even by the saliva diastase, that pepsin was less injurious, that trypsin had considerable action upon it, and that bile in large quantity completely destroyed it. He could not detect any influence of the intestinal mucous membrane and the intestinal bacteria upon the poison, although Fermi and Pernossi had found it to be very pronounced. Nencki and Schoumow-Simanowski[a] assert that the digestive fluids render it completely innocuous, notably the bile in conjunction with the fluid from a pancreatic fistula, while trypsin alone has less effect, being less injurious than pepsin. Vincenzi,[b] on the other hand, states that *normal* bile has hardly any destructive influence, but that the bile of animals infected with tetanus has a slight effect under certain conditions; thus, for example, death inevitably follows in three to four days when the bile is active. On the other hand, the poison is somewhat weakened by the *oxydase* of the leucocytes, and, according to Sieber,[c] is completely destroyed by the oxydase of the spleen.[d]

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[a] Ransom, F.: Das Schicksal des Tetanusgiftes nach seiner intestinalen in den Meerschweinorganismus. Deu. med. Woch., vol. 24, 1898, pp. 117 118.

[b] Carrière: "Toxines et digestion." Ann. Past., xiii, 435, 1899 gives a bibliography of the subject), cf. the General Part.

We have fed guinea pigs as much as 24,000 and mice 18,000 times the M. L. D. of tetanus toxine (if injected subcutaneously) without producing any apparent ill effects.

Up to the present time all attempts to isolate the specific toxine as a definite chemical compound have proved unavailing; we are totally ignorant of its chemical nature. Animal experiments are the only real agents by which it may be recognized. By this means we are enabled to determine not only the presence of the poison, but also to estimate its concentration in a solution and to follow its changes.

Before the tetanus bacillus was obtained in pure culture the chemical nature of the toxine was studied by Brieger from mixed cultures in meat broth obtained from Nicolaier and Rosenbach. In 1886 Brieger[e] isolated from such a contaminated growth a basic substance or ptomaine which he called *tetanine* ($C_{13}H_{30}N_2O_4$), and which caused muscular contractions when injected into mice.

Shortly afterward Brieger[f] obtained another ptomaine which he named *tetano-toxine* ($C_5H_{11}N$). He believed these to be the true poison of tetanus. These are now only of historical interest.

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[a] Nencki and Schumow-Simanowski: "Ueber die Entgiftung der Toxin durch die Verdauungssäfte." Centralb. f. Bakt., xxiv, 84. cf. Dzierzgowski and Sieber. Archiv des Siences Biol. de St. Petersb., viii.

[b] Vincenzi: "Ueber antitoxische Eigenschaften der Galle tetanisierter Tiere." Deutsch. med. Woch., 1898, 534.

[c] Sieber: "Ueber d. Entgiftung der Toxin durch die Superoxyde, etc." Z. f. phys. Ch., xxxii, 573, 1901.

[d] Toxines and Antitoxines; Oppenheimer, Carl, trans. by C. Ainsworth Mitchell. Charles Griffin & Co., Ltd., London, 1906, p. 110.

[e] Brieger, L.: Ueber ein neues Krämpfe verursachendes Ptomain. Berichte d. deut. chem. Gesellsch., vol. 19, Dec. 9, 1886, p. 3119.

[f] Brieger, L.: Zur Kenntniss der Aetiologie des Wundstarrkrampfes, nebst Bemerkungen über das Choleraroth. Deut. med. Woch., vol. 13, 1887, p. 303.

———: Ptomaine. 3 bd., 1886, p. 89.

———: Ueber ein neues Krämpfe verursachendes Ptomain. Berichte d. deut. chem. Gesellsch., vol. 19. 1886. p. 3119.

Knud Faber,[a] in 1890, first succeeded in demonstrating by physiological tests upon animals the poison in the filtrate of mixed cultures; but exact work upon the toxine dates from Kitasato's anærobic method, in 1889 and 1891, of growing the tetanus bacillus in pure culture upon solid and fluid media.

Brieger's experiments were repeated by Kitasato and Weyl[b] in 1890 with pure cultures of tetanus. From their studies they concluded that Brieger's tetanin, etc., did not produce the characteristic symptoms of tetanus in experimental animals.

Brieger and Fränkel,[c] in 1890, in further experiments believed the tetanus toxine, as well as the poisons produced by the whole order of important pathogenic micro-organisms, to be "toxalbumins." Brieger and Fränkel's toxalbumin consisted practically of an alcoholic precipitate from filtered broth cultures and was undoubtedly toxic.

Hayashi[d] concludes, from his work upon the subject, that the toxine isolated according to the Brieger-Boers method, as well as by his own modification, shows a definite albumin reaction. However, this is not proof that the toxine is a proteid.

The powerful action of tetanus poison in minute amounts, its thermolability, and the period of incubation lend countenance to the view that the toxine may be a ferment. But ferments continue to act when the products of fermentation are removed. This has not been shown to be the case with tetanus toxine. Many of the higher albuminous substances are also readily destroyed by heat lower than 65° C., which is fatal for the tetanus toxine. The period of incubation is now explained by the time necessary for the poison to travel up the motor nerves to the centers. There is, therefore, nothing but analogy of an indefinite nature to class tetanus toxine with the ferments.

Ehrlich,[e] in a parallel work to his researches upon the constituents of the diphtheria toxine, showed that the tetanus toxine contains both a toxophore and a haptophore group, and that the antitoxic immunity is explained by the presence of free receptors in the blood of immunized animals. The receptors combine directly with the haptophore group, thus neutralizing the toxine.

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[a] Feber, Knud: Die Pathogenese des Tetanus. Berl. klin. Woch., vol. 27, 1890, pp. 717–720.

[b] Kitasato, S., and Weyl, Th.: Zu Kenntniss der Anaeroben. Zeit. f. Hyg., vol. 8, 1890, pp. 41, 404.

[c] Brieger, L., and Fränkel, Carl: Untersuchungen über Bakteriengifte. Berl. klin. Woch., vol. 27, 1890, p. 268.

[d] Hayashi, H.: Weitere Forschungen über die chemische Natur des Tetanustoxins. Arch. exper. Path. u. Pharm., vol. 47, 1901–1902, pp. 9–18.

[e] Ehrlich, P.: Die Werthbestimmung des Diphtherieheilserums. Klin. Jahrb., 1897.

———: Ueber die Constitution des Diphtheriegiftes. Deut. med. Woch., vol. 24, 1898.

Ehrlich,[a] 1898, definitely proved that tetanus toxine contains at least two poisons—(1) *tetano-lysin* and (2) *tetano-spasmin*. He showed that these two poisons do not always appear in the same relative proportion in different preparations. Some of the toxines that have strong tetanic properties have weak hemolytic action, and *vice versa*. The hemolytic affinity of the toxine weakens much quicker than the tetano-spasmin. This occurs spontaneously as well as when it is heated to 50° for twenty minutes. The two poisons have different combining affinities. If tetanus toxine is brought into contact with red blood corpuscles, the greatest part of the tetanolysin is bound by the red corpuscles, while the tetanospasmin remains in the solution. Each one of these two poisons has its own antitoxin. If several different tetanus sera are examined, it will be found that they have no parallel neutralization for tetanolysin and tetanospasmin. In one particular case Ehrlich found a serum that was strongly antispastic and had practically no antilytic power.

Madsen,[b] 1899, confirms Ehrlich's observations that in cultures of the tetanus bacillus there are two poisons, namely, tetanolysin and tetanospasmin, and that for the former there exists a specific antitoxin, namely, antilysin. The strength of this lysin and its antibodies may be measured with great accuracy by colorimetric tests.

Knud Faber,[c] in his early work with mixed cultures in 1890, was the first to obtain indications of the true tetanus poison.

Faber showed that the poison always works after a certain period of incubation. The greater the quantity of poison the shorter the period of incubation and the shorter the course of the disease. For instance, when the period of incubation was twenty-four hours, rabbits died after a sickness of twenty-four hours; but if the period of incubation was five days, then the rabbits recovered after localized tetanus lasting fourteen days. With mice the period of incubation varied from six hours to two to fifteen days.

He found that the poison is active not only when injected into the tissues, but also when injected intravenously in rabbits. Positive results were obtained with mice, rats, guinea pigs, rabbits, and birds, but frogs were not affected.

He was not able to obtain an immunity by using the filtered cultures. The poison passes through a Chamberland filter. All traces of toxicity disappear if the poison is heated for five minutes at 65° C. Faber states that experimental tetanus in smaller animals always manifests itself by local cramps. This is the case whether the toxine or a bacterial culture is used; so that we must conceive that the disease spreads in and around the wound, working upon the motor nerve endings (?). The general spasms are produced by the introduction of the poison into the blood.

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[a] Ehrlich, P.: Gesellschaft der Charité-Aertze (Sitzung 3. Februar 1898). Berl. klin. Woch., 1898, no. 12.

[b] Madsen, Thorvald: Ueber Tetanolysin. Zeit. f. Hyg., vol. 32, 1899, pp. 214, 239.

[c] Faber, Knud: Die Pathogenese des Tetanus. Berl. klin. Woch., vol. 27, 1890, pp. 717–720.

In 1891 Kitasato,[a] working under Koch's directions, published his classical paper upon tetanus toxine. The toxine was readily obtained in any desired amount by simply filtering pure cultures of the organism.

Of the three species of animals used in these experiments—mice, guinea pigs, and rabbits—Kitasato found that guinea pigs are most susceptible to tetanus toxine; then follow mice, and finally rabbits. The symptoms of tetanus in all three animals injected with the filtrate appear at the latest in three days. If the animals remain well for four days they will not sicken with tetanus.

The tetanus toxine appears in the blood and there produces its toxic properties. The blood itself is sterile, and therefore it is a toxic and not a bacterial result. The bouillon must be neither acid nor strongly alkaline, but about neutral or have a weak alkalinity; and, secondly, it must always be freshly prepared.

Meyer[b] injected dogs and cats with tetanus toxine into the spinal cord and found the condition known as tetanus dolorosus. This is never found in any other method of tetanus poisoning. By injecting the posterior roots of the spinal cord in the lumbar region Meyer obtained a pure tetanus dolorosus without muscular contractions.

Bolton and Fisch[c] have shown that the toxine makes its appearance in the blood of the horse several days before any symptoms of tetanus are to be observed, and that it gradually increases until about two days before symptoms become noticeable, and then it suddenly diminishes, and even disappears in some cases. The amount of toxine varies considerably in different cases. In one instance the serum from a diphtheria antitoxin horse about two days before symptoms of tetanus appeared was sufficient to kill a guinea pig in the dose of 0.1 c. c. The fact that tetanus toxine may appear in such large quantities in the blood without symptoms of tetanus is of very great practical importance in the production of both diphtheria and tetanus antitoxins.

Von Lingelsheim[d] recommends that in order to obtain strong tetanus toxines only those cultures which are strictly anaërobic should be used. According to Debrand,[a] the strongest poisons may be obtained by symbiosis with *B. subtilis*. The best medium is a weak alkaline bouillon with 1 per cent peptone and 0.5 per cent salt. The addition of glycerin and grape sugar is to be avoided, as the acids produced by their decomposition have an injurious effect upon the toxine. Tizzoni obtained good results by the addition of gelatin to the culture medium. It is most important to have a culture which is a good toxine producer, just as in the case of diphtheria. It seems that the strongest poisons are obtained after a term of from six to eight days in the incubator. By observing the proper conditions it seems comparatively easy to obtain cultures that will kill a mouse weighing 15 grams in a dose of 0.000005 gram. Most of those who have worked much in this subject have experienced at times difficulty in obtaining strong tetanus toxine; not all of the factors involved are understood.

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[a] Kitasato, S.: Experimentelle Untersuchungen über das Tetanusgift. Zeit. f. Hyg., vol. 10, 1891, pp. 267–305.

[b] Meyer, Hans: Ueber motorischen und dolorosen Tetanus. Sitzber. d. Gesellsch. z. Bef. d. Naturw., Marburg, 1902, pp. 1–6.

[c] Bolton, B. Meade, and Fisch, Carl: An estimate of the amount of toxine in the blood of horses infected with tetanus. Trans. Assn. of Am. Phys., vol. 17, 1902, pp. 462–467.

[d] Von Lingelsheim, W.: Das Tetanusgift. Kolle & Wassermann's Handbuch der Bakterien, Bd. 2, p. 589.

It is noteworthy that the strength of the toxine produced is greater in contaminated cultures than in pure cultures. In the Marburg Institute of Hygiene and Experimental Therapy, presided over by Behring,[b] the strongest tetanus toxines have been obtained from mixed cultures. The first work, it will be remembered, upon the tetanus toxine by Faber, Brieger, Fraenkel, Nicolaier, and Rosenbach was with impure cultures.

Uschinsky[c] cultivated tetanus bacilli on a proteid-free medium of the following composition:

Water	1,000
Glycerin	30 to 40
Sodium chloride	5 to 7
Calcium chloride	0.1
Magnesium sulphate	0.2 to 0.4
Dipotassium hydrogen phosphate	2 to 2.5
Ammonium lactate	6 to 7
Sodium aspartate	3.4

preferably with the addition of 1 to 2 per cent of grape sugar. The air was excluded by means of liquid paraffin.

According to Buchner,[d] Tizzoni and Cattani, following the plan of Brieger and Fränkel, were the first to succeed in obtaining a dried poison with the use of ammonium sulphate.

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[a] Debrand, L.: Sur un nouveau procédé de culture du bacille du tetanos. Ann. d. Inst. Pasteur, vol. 14, 1900, pp. 757–768.

[b] v. Behring, E.: Aetiologie und aetiologische Therapie des Tetanus. Beitr. z. exper. Therapie, vol. 7, 1904, pp. 1–72.

[c] Uschinsky, "Ueber eine eiweissfreie Nährlosung f. pathog. Bakt." Cantralb. f. Bakt., vol. 14, 1893, p. 316.

[d] Buchner, H.: Erwiderung betreffend das trockene Tetanusgift. Deut. med. Woch., vol. 20, 1894, p. 179.

Brieger and Fraenkel,[a] however, call attention to the fact that in 1890, in the Berliner klinische Wochenschrift, No. 11, page 612, they describe a process for obtaining a dried tetanus poison and experiments with the same.

Whoever owns priority, the method is a very good one, and with it we have succeeded in obtaining strong and stable poisons.

MODE OF ACTION OF TETANUS TOXINE.

For a full understanding of the problem with which we are dealing it is important to have a clear conception of the way in which the tetanus toxine exerts its poisonous effects upon the body. Therefore the following review of the literature upon this question has been introduced.

It has been known since ancient times that tetanus toxine affects chiefly the central nervous system, but it is only comparatively recently that it has been experimentally demonstrated in what way the toxine reaches the nervous centers.

Gumprecht[b] first showed with some degree of certainty that the toxine attacks particularly the spinal cord and that the phenomenon of the disease depends upon this cord poisoning.

Bruschettinni,[c] 1892, demonstrated the presence of the toxine in the central and peripheral nervous system after subcutaneous inoculation. He also showed it to be present in the blood, but that the other organs were free from it.

Brunner,[d] 1892, in experiments upon guinea pigs and rabbits, found that the tetanus poison was not able to cause the tetanic contractions when placed directly upon the muscle substance. After paralysis of the motor nerve terminals by curare the tetanus contractions ceased. He found, however, that the tetanus toxine is not able to cause spasms in a muscle area the motor nerve of which has been severed from its center. In view of the first fact, it followed that a motor nerve severed from its center is not able to produce tetanus contractions.

Brunner further concluded that the poison was able to induce tetanic spasms in the muscles of a particular nerve area only when the paths to the corresponding nerve centers are intact.

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[b] Brieger, Ludwig, and Fraenkel, Carl: Herr Buchner und das trockene Tetanusgift. Deut. med. Woch., vol. 20, 1894, p. 118.

[b] Gumprecht: Versuche über die physiologische Wirkung des Tetanusgiftes im Organismus. Arch. f. Physiol.

[c] Bruschettinni, Alessandro: Sulla diffusione del veleno del tetano nell'organismo. La riforma med., vol. 8, part 3, 1892, pp. 256–259.

[d] Brunner, Conrad: Zur Pathogenese des Kopftetanus. Berl. klin. Woch., 1891.

———: Die bisherigen Resultate experimenteller Untersuchungen über die Art der Wirkung des Tetanusgiftes auf das Nervensystem. Deut. med. Woch., vol. 20, 1894, pp. 100–103.

Vaillard and Vincent,[a] 1890, had already shown that the contractions in a particular innervation of the area disappeared when such an area was cut off by section of the cord.

Knorr,[b] 1897, as a result of his work, concluded that:

> Pure tetanus poison has a strong affinity for some unknown substance in certain of the body cells. The amount of this affinity is as the degree of susceptibility of animals (that have not previously been treated) for the poison.
>
> The antitoxin has the same property of combining with the toxine as has the above-mentioned unknown substance.
>
> In the culture media of tetanus bacilli there are also sometimes found bodies that have an affinity for the poison.
>
> The combination of the poison with all of these substances is slow. The rapidity depends upon the degree of the affinity and the concentration, and other conditions surrounding the poison.
>
> For every animal species the dose most favorable for the production of antitoxin seems to be slightly less than that necessary to make the animal sick. Doses that are much smaller are not effective. Doses that are considerably higher interfere with the production of antitoxin. The production of antitoxin begins very soon after the inoculation of a favorable amount of toxine.
>
> The antitoxin is a product of the body and appears to be a specific substance of the cells.

The tetanus toxine combines chemically, according to Milchner,[c] 1898, with the antitoxic group of the central nervous system in the test tube, without the help of the living organism. This indicates, according to Ehrlich's theory, that there is also a chemical union between the toxine and the nervous system in the living body.

Marie,[d] 1898, showed that the toxine injected into animals remains a variable time in the blood; this time passed, the inoculation of the organs and glandular secretions is negative. When injected under the skin, a part of the toxine is taken up by the nerve fibers. He also found that the brain of rabbits neutralizes tetanus toxine. The cells of the central ganglions are much less active than those from the cerebral surface.

For the purpose of testing the action of cord and brain of normal animals on tetanus toxine, Wassermann and Takaki,[e] 1898, used a solution of tetanus toxine, containing as a preservative an equal amount of glycerin, and of such strength that 0.001 c. c. was a fatal dose to a mouse weighing 15 grams within three days after inoculation.

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[a] Vaillard and Vincent: Sur. le poison tétanique. La sem. med., vol. 10, 1890, pp. 425–426.

[b] Knorr, A.: Die Entstehung de Tetanusantitoxins im Theirkörper und seine Beziehung zum Tetanusgift. Fortschr. d. Med., vol. 15, 1897, pp. 657–669.

[c] Milchner, Richard: Nachweiss der chemischen Bindung von Tetanusgift durch Nervensubstanz. Berl. klin. Woch., vol. 35, 1898, pp. 369–371.

[d] Marie, A.: Recherches sur les la toxine tétanique. Ann. Inst. Pasteur, vol. 11, 1897, p. 591; vol. 12, 1898, p. 91.

[e] Wassermann, A., and Takaki, T.: Ueber tetanusantitoxische Eigenschaften des normalen Centralnervensystems. Berl. klin. Woch., vol. 35, 1898, pp. 5–6.

They mixed a single, double, triple, ten times fatal dose, etc., of the tetanus toxine with 1 c. c. of emulsion of normal cord or brain in a flask and injected this subcutaneously in the dorsal region. As controls, equal amounts of emulsion of liver, kidney, spleen, marrow, or 1 c. c. serum of same animal were mixed with the specified quantities of toxine and likewise subcutaneously injected into mice, and as a further control the tetanus toxine alone was injected. In all their experiments thus carried out they could, without exception, demonstrate that normal brain or cord always had an antitoxic action, while no other tested organ showed this action.

The antitoxic power of the brain of normal guinea pigs is so decided that 1 c. c. of brain emulsion will neutralize ten times the lethal dose. A definite retarding influence is exerted by the emulsion on a 60-times lethal dose. The cord is definite, though feebler, in its influence; its emulsion will neutralize three times the fatal dose.

They were able to immunize against three and five times the fatal dose by an injection of emulsion of brain twenty-four hours before. They could even save a mouse that had received a fatal dose of toxine by the injection of the emulsion four to six hours after. The tetanus toxine-binding power of the central nervous system acts not only by direct mixing in vitro, but also in the living organism. Their experiments lead them to believe that the fixing power of the central nervous system is due to a property of the cell and not to a water-soluble substance.

Metchnikoff,[a] 1898, agrees with Wassermann and Takaki that the brain and cord of guinea pigs neutralize tetanus toxine. From his own experiments he concludes that the antitetanic action of the nervous centers is a property of mammals. The nervous centers of the fowl are much less efficacious, the tortoise only very feebly so, and the frog not at all.

Roux and Borrel,[b] 1898, find that animals are much more sensitive to intracerebral than to subcutaneous injections of toxine. Animals highly resistant to injections under the skin or in the veins are sensitive to injections into the brain. The nerve cells are still sensitive to the toxine.

Danysz,[c] 1898, showed that:

> The cerebral substance of guinea pigs fixes a variable amount of tetanus toxine according to the medium used.
>
> The quantities of toxine fixed by the cerebral substance are much greater when the liquid itself in which the emulsion has been made, exercises the least energetic modify-

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[a] Metchnikoff, E.: Sur l'influence de l'organisme sur les toxine. Ann. Inst. Pasteur, vol. 12, 1898, p. 81.

[b] Roux, E., and Borrel, A.: Tétanos cérébral et immunité contre le tétanos. Ann. Inst. Pasteur, vol. 12, 1898, p. 225.

[c] Danysz: Contribution a l'étude de la toxine tétanique. Ann. Inst. Pasteur, vol. 13, 1898, pp. 156–168.

ing action on the toxine. This quantity is the greatest in physiologic salt solution, which best preserves the cerebral substance in its normal state. If the amount fixed by the brain emulsified in normal salt solution be taken as 100, we will have for the others about the following proportions:

If a brain of a guinea pig emulsified in normal salt solution absorbs 100 parts toxine, the same brain emulsified in distilled water will absorb 90 parts toxine; if emulsified in 10 per cent salt solution, will absorb 5 parts toxine; if boiled in distilled water, will absorb 10 parts toxine.

The fixation of the toxine by the cerebral substance is not permanent, as has been previously shown by Metchnikoff. It diffuses in the liquid of maceration, and this diffusion is much more rapid when the liquid exercises a more energetic action upon the nervous substance.

He concluded that the phenomenon of the fixation of the tetanus toxine by nervous tissue, in spite of some analogies, can not be likened to the action of antitoxin on toxine, because the toxine at first fixed by the nervous substance becomes again free *in vitro* and *in vivo*. The neutral mixture of toxine and nervous substance becomes again with time more and more toxic, while, on the contrary, the toxic mixture of toxine and antitoxin become with time less toxic.

Blumenthal and Jakob,[a] 1898, used a method to free a part of the tetanus toxine which has been fixed in the central nervous system. They concluded that the tetanus poison at the time of the outbreak of the symptoms of tetanus is so anchored in the central nervous system that it can not be set free by means of subdural infusions of antitoxin.

Ransom[b] found that the tetanus toxine as well as the antitoxin is taken up from the subcutaneous tissue by means of lymphatic vessels and carried to the blood stream.

After intravenous injection a considerable portion of the toxine as well as the antitoxin is carried over into the lymph. In the cerebro-spinal fluid, on the other hand, the antitoxin appears in comparatively small amounts and the toxine can not be definitely demonstrated. Tetanus antitoxin injected into the subarachnoid space does not cause an increase in the normal, detoxicating power of the nerve substance and most all of it quickly passes over into the blood stream.

After injections of the toxine into the central nervous system the nervous system becomes toxic itself and loses for a time the property of neutralizing the poison. This is not due to adherent cerebro-spinal fluid, but rather to the fact that the nerve substance holds the toxine fast.

The disappearance of tetanus toxine from the blood of chickens after intravenous injections of small quantities does not depend upon the combination of the unchanged toxine in any one organ. Up to the time when the toxine disappears and the antitoxin begins to appear in the blood no collection of antitoxin can be found in any organ. The intravenous injection of very small quantities of toxine into hens, quantities too small to cause harmful reactions, causes considerable antitoxin to appear.

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[a] Blumenthal, Ferdinand, and Jakob: Zur Serumtherapie des Tetanus. Berl. klin. Woch., 1898.

[b] Ransom, F.: Die Vertheilung von Tetanusgift und Tetanusantitoxin in lebenden thierischen Körper. Berl. Klin. Woch. vol. 38, 1901, pp. 337-340; 373-375.

The above experimental work on the affinity of the nerves for the toxine and the neutralization of the toxine by the nervous tissue led Marie and Morax,[a] 1902, and Meyer and Ransom, 1903, independently to study the method of absorption of the toxine. The paper by Marie and Morax was published in 1902. They find that the absorption of tetanus toxine by the peripheral nerves is in consequence of the specific affinity for the axis cylinder substance. This affinity is not shown in experiments *in vitro*, contrary to those which are observed with the cord or with the brain, but is shown with the greatest ease by experiments *in vivo*. Fixation of the toxine upon the nerves occurs very rapidly. It has the characteristic of not being very stable.

They showed that the toxine once fixed, is displaced centripetally with a relatively feeble rapidity. This axis cylinder circulation has for its effect the transportation of the diluted toxine in the fluids to the ganglionic cells. The authors believe that they are able to formulate the following hypothesis: Injected into a muscle or into a region a little removed from a muscular mass, the toxine distributes itself in the serous fluid which impregnates the tissues, and passes in part to the blood, where it can be found very soon. In the zone of inoculation the serous fluid charged with the toxine is found in contact with expansions of the nerves at their endings. The motor nerves as well as the vaso-motor nerves absorb it and transfer it, so that in a relatively short time the substance of the peripheral nerves contains it in quantity larger than the fluids which bathe the tissues at some distance from the point of absorption. Diffusion of the toxine occurs centripetally. It may be compared to the absorption of nourishing liquids by the roots of a plant. The portion of toxine contained in the lymph at the point of contact remains twenty-four hours or less. This amount is greater than the portion contained in the blood, and consequently in the lymph in different parts of the body. Absorption by the nerve fibers at the region inoculated is then greater during the first twenty-four hours than that which takes place in other regions. The corresponding motor nerves will be, consequently, the first saturated by the toxine, and this saturation will be manifested by a localized contraction or by a local tetanus. However, in other regions the terminations of the nerves will absorb a certain quantity of toxine carried to them by the lymph. It is then that local tetanus will be succeeded by general tetanus. We are not yet able to decide whether the tetanus toxine can pass from the peripheral neuron to a sensory neuron with which it is connected, or if the contractions are to be attributed to the action of the toxine upon the one or the other of those cellular elements.

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[a] Marie, A., and Morax, V.: Recherches sur l'absorption de la toxine tetanique. Ann. Inst. Pasteur, vol. 18, 1902, p. 818-832.

In the absorption of the toxine it is necessary to consider not only the number of absorbing elements, but also the concentration of the solution of the toxine, for this concentration is always much more considerable at the point of inoculation than in other regions, where the toxine only diffuses in the mass of blood. This preponderating absorption by the nerve fibers at the inoculated region explains why the injection at several points of the body of a nonfatal dose of the toxine provokes the appearance of tetanus earlier and more severely than inoculation at a single point. When, instead of injecting the toxine under the skin or into the muscle, it is introduced directly into a viscus, the symptoms of tetanus appear only when the motor nerves are bathed in the toxine circulating in the blood.

In a later paper Marie and Morax[a] found that the central nerve substance of mammals, which possesses the power of neutralizing tetanus toxine, loses its power almost completely by drying in a vacuum. Some brain of a guinea pig finely bruised up is exposed under a vacuum where it loses about three-fourths of its weight. When they endeavored to repeat the experiments of Wassermann with this cerebral powder it was found that the nervous tissue had lost 97 per cent of its fixative power. This fixative power is not influenced by a dry heat of 60°, 100°, or 126° C. It is known, on the other hand, that the drying of antitetanic serum causes slight modification. The phenomenon of Wassermann would not seem, then, to be similar to the antitoxic action of specific sera.

The work of Meyer and Ransom[b] was done about the same time as the work referred to above by Morax and Marie, but not published till July, 1903. They found that the toxine is carried toward the centers by the motor nerves, and that this is the only way for the poison to reach the central nervous system. The toxine absorption by the nerve is dependent upon the integrity of the axis cylinder. A normal nerve retains the toxine for an hour and a half after intramuscular injection, a torn nerve about twenty-four hours; but if the nerve is degenerated it does not absorb the poison till about six hours later. If the nerve be cut before inoculation, there is no toxine in the proximal portion, but it is present in the distal portion. The toxine therefore enters the nerve through the muscle plates and not by the capillaries. After tearing a poisoned nerve the toxine disappears in a few hours in the proximal end, as the rupture stops the upper current. The passage of the toxine upward in the spinal cord is barred by section of the cord. The period of incubation is much shorter when the toxine is injected into the nerve than after subcutaneous or intravenous injections; injections into the cord have a still shorter period of incubation.

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[a] Morax, V., and Marie, A.: Note sur les propriétés fixatrices de la substance cérébrale desséchée. Bull de l'Inst. Pasteur, vol. 1, 1903, p. 41.

[b] Meyer, H., and Ransom, Fred.: Untersuchungen über den Tetanus. Arch. f. exper. Path. u. Pharm., vol. 49, 1903.

Their theory of tetanus is that the toxine is probably absorbed by the axis cylinder endings and reaches the corresponding spinal nerve center by that route; from the spinal center it spreads to the corresponding center in the other half of the cord and outwards, resulting in generalized tetanus. When intoxication is produced through the circulation, the poison is taken up by the nerve ends in all parts of the body and the disease is not localized, but general. The antitoxin, unlike the toxine, does not travel by the nerve route, but is found only in the blood and lymph.

Besredka[a] showed that the cerebral mass is capable of fixing more tetanus toxine than it is capable of neutralizing. The fixative substance of the brain is not then an antitoxic substance in the proper meaning of the word. The cerebral mass saturated with tetanus toxine recovers some of its primative integrity after the addition of true antitoxin, whether this comes from an animal of the same species or of a different species. The combination of the brain and toxine does not represent, then, a stability comparable to that which exists between the toxine and its true antitoxin. The phenomenon observed by Wassermann and Takaki ought then to be attributed to the presence in the cerebral matter of a particular substance other than the true tetanus antitoxin.

Dmitrievsky,[b] 1903, found that the brain of animals in which the immunization has been of only short duration and of which the blood does not possess high antitetanic power does not differ in its antitetanic property from a normal brain. The brain of animals which were immunized for a long time and of which the blood contained a large quantity of antitoxin is able to inactivate a much larger quantity of tetanus toxine than the brain of normal animals. This difference, however, is not considerable, for all the animals injected with the mixture of the poison and of brain from a guinea pig immunized presented some slight tetanic symptoms. The blood of animals of which the immunization has been continued a long time contains always more tetanus antitoxin than their brain.

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[a] Besredka, A.: De la fixation de la toxine tétanique par le cerveau. Ann. Inst. Pasteur, vol. 17, 1903, pp. 138–147.

[b] Dmitrievsky, K.: Recherches sur les propriétés antitétaniques des nerveaux de l'animal immunisé. Ann. Inst. Pasteur, vol. 17, 1903, pp. 148–160.

A second paper by Morax and Marie,[a] appeared in 1903, in which they showed that the affinity of tetanus toxine for the nerves seems to be shown *in vitro* by the experiments of Wassermann and is proved *in vivo* by the presence of this poison in the nerves of tetanized animals. It has been definitely established that it is the peripheral expansions of the neurons which absorb the toxine for transportation from point to point toward the nerve cell.

The object was to determine the respective neurons in this fixation and in this transportation of the toxine. The discovery of the poison in the nerve areas has shown that the three different types of nerve fibers constituting the cord, viz, the motor fibers, sensory, and sympathetic, are equally able to absorb the tetanus toxine. The considerable portion of this poison found in the massateric nerves is in conformity with the early appearance of tetanus in this region. The total absence of tetanus toxine in the optic nerves corroborates the anatomical findings which show them to be a prolongation of the brain, as it is known that the toxine is not found in the medulary neurons or brain, since it is fixed by them.

Blumenthal[b] concludes that:

> The cells of the body as well as the central nervous system have the property of binding tetanus toxine.
>
> The tetanus poison is altered in the organism as a consequence of this binding with the cells.
>
> There is an affinity of the antitoxin for the tetanus poison which has been bound in the tissues. This affinity is, however, comparatively slight in comparison to that it has for the toxine secreted by the cultures.

Tiberti,[c] 1905, concludes as follows:

> If we inject tetanus toxine subcutaneously into susceptible animals, the largest part first goes into the lymphatic vessels and from there into the blood. A smaller quantity is absorbed by the nerve endings and is carried by them to the nerve centers. After hypodermic injection of tetanus toxine in a limb the toxine-constant in the large nerves of that limb is demonstrable.
>
> The transportation of the tetanus toxine to the nerve centers by the nerve routes takes place not through the lymph channels, but in the plasma of the nerve fiber consisting of the axis cylinder. In order that the nerve fibers may be capable of taking up the tetanus toxine and conducting it to the nerve centers, it is necessary that the axis cylinder be normal.
>
> The tetanus toxine moves toward the nerve cells because the latter have a special affinity for tetanus toxine and in that way the nerve fibers become saturated.
>
> If tetanus toxine is injected into a muscle it diffuses throughout the muscle itself in the serous fluid. It is then taken up by the nerve endings in the muscle and by means of the nerves is carried to the center.

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[a] Morax, V., and Marie, A.: Recherches sur l'absorption de la toxine tétanique. Ann. Inst. Pasteur, vol. 17, no. 5, 1903, pp. 335–342.

[b] Blumenthal, Ferdinand.—Ueber das an die Organe gebundene Tetanusgift und seine Beziehung zum Antitoxin. Arch. f. physiol., 1904, pp. 217–220.

[c] Tiberti: Ueber den Transport des Tetanusgiftes zu den Rückenmarkszentren durch die Nervenfasern. Centblt. f. Bakt., 1 abt, vol. 38, 1905, pp. 281, 413, 499, 625.

Tetanus toxine inoculated into the gluteus muscle of a guinea pig is found in the corresponding ischiatic nerve in evident doses one and a half hours after the injection, whereas it is found much quicker in the blood, viz, after ten minutes.

Minimal doses of tetanus toxine are sufficient to cause severe symptoms of tetanus poisoning if injected into the parenchyma of a nerve. The same doses if injected under the skin or into the circulation produce no tetanic symptoms.

If tetanus antitoxin is injected into a nerve trunk and immediately thereafter tetanus toxine is inoculated into the corresponding muscle enervated by this nerve, it is possible to prevent the toxine reaching the corresponding nerve center and no symptoms of tetanus of any kind appear in the muscle area in question.

Tetanus toxine inoculated directly into the nerve has no other channel of transference to the nerve centers than the nerve substance itself. This fact is demonstrated by severing the spinal cord at a particular point, in which case the effect of the toxine is limited to the area corresponding to the section of the cord connected with the seat of the inoculation. And, further, the section of the spinal cord prevents the spreading of the toxine to the upper regions of the cord.

By cutting a particular nerve trunk the muscles innervated by this nerve show no indication of tetanus following the inoculation of tetanus toxine.

If tetanus antitoxin is injected directly into the substance of the spinal cord, then we see a decided abbreviation of the period of incubation, and such inoculation gives us a characteristic picture of the disease known as tetanus dolorosus.

If tetanus toxine is injected into the circulation, we have, after a longer or shorter period of incubation, dependent upon the animal species, all muscles simultaneously seized with tetanus contractures, because the tetanus toxine is absorbed by all the nerve trunks at the same time and thus conveyed to the nerve centers. In such cases we do not get the so-called local tetanus which is observed when the toxine is injected under the skin or into the parenchyma of the nerve. It requires a much stronger dose of toxine in order to produce symptoms of tetanus in an animal when the toxine is injected directly into the circulation than when it is given subcutaneously or directly into a nerve.

The tetanus toxine injected into the circulating blood quickly passes out into the lymph. The tetanus toxine can not be demonstrated with certainty in the cerebrospinal fluid.

Loew and Meyer[a] say that the characteristic symptoms of tetanus poisoning are to a certain extent due to the effects of the toxine upon the central nervous system. An exception was found by Dönitz in the condition called by him "tetanus sine tetano." He found that in intravenous injections in rabbits of tetanus toxine in quantities insufficient to produce local tetanus the animals nevertheless rapidly lost weight and died. Dönitz believed that the cause of cachexia was a parenchymatous degeneration. Loew and H. Meyer injected a number of rabbits with small quantities of tetanus toxine after the manner of Dönitz and found that some of them died without symptoms of tetanus and that some remained well. The animals that died showed symptoms of coccidial infection, and he therefore concluded that the cachexia was not caused by the tetanus toxine.

Loew and Meyer then conducted some experiments to determine whether animals could be rendered hypersusceptible to tetanus poison in the same sense that Behring and Kitashima showed in guinea pigs treated with small doses of diphtheria toxine. These investigations terminated negatively. The authors succeeded, however, in inducing hypersusceptibility to tetanus toxine in rabbits by the intradural injection of minute amounts.

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[a] Loew, O., and Meyer, H.: Zur Kenntniss der Tetanusvergiftung. Sitzungsb. d. Gesellsch. f. Beförd. d. ges. Naturw. zu Marb. (1904), 1905, p. 11-13.

From the above review it seems to have been definitely shown that the motor nerves have a specific affinity for tetanus toxine. When the toxine is given subcutaneously, the adjacent motor nerve-endings at once begin to take it up and transport it to the cord. The lymphatics also absorb much of it, and in a short while it appears in the blood stream, which carries it to all parts of the body, where it is absorbed by the motor nerve-endings, which are bathed in the toxine-laden fluid. The reason that the symptoms appear earlier in the limbs in which the toxine is injected is because the toxine-index, as it were, is higher there than in other parts of the body. When the toxine is given into the cord or into a nerve trunk, the incubation period is shortened; if into a nerve trunk, the tetanus is sharply localized at first, and in fact the animal may die before general tetanus sets in. When given into the circulation, generalized tetanus results in a short time. The weight of evidence is in favor of the view that the toxine produces its effect solely by the absorption of the toxine by the nerves, whose axis cylinder substance has a specific affinity for the poison. They transport it to the cord and brain through the axis cylinder substance itself.

THE GERMAN METHOD OF STANDARDIZING TETANUS ANTITOXIN.[a]

The official testing of this serum is conducted at the Kgl. Institut für experimentelle Therapie zu Frankfurt a. M., of which Dr. Paul Ehrlich is the director. Tetanus antitoxine, like other curative sera, is examined in the Institut first for harmlessness and second for potency.

The serum is considered harmless if (1) it is wholly clear and free from much precipitate; (2) contains no bacterial contamination; (3) contains not more than 0.5 per cent phenol or 0.4 per cent trikresol; (4) is free from toxines, especially tetanus toxine.

The serum should not contain more than a very slight precipitate at the bottom and must under all circumstances be absolutely clear. The tests of the serum for sterility are made in accordance with the usual bacteriological methods. Five drops of the serum are planted into one agar plate, two glucose-bouillon test tubes, and a glucose-agar test tube with a sufficient quantity of the media for deep anærobic cultures. The inoculated plates and tubes must remain sterile. In order to test the quantity of phenol contained in the serum, a mouse weighing 15 grams is injected subcutaneously with 0.5 c. c. of the serum. If this mouse shows slight or no symptoms of poisoning, then the serum does not contain an excessive amount of the preservative allowed.

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[a] The account of the German methods of testing is taken from Otto's work entitled "Die Staatliche Prüfung der Heilsera." Jena, 1906. Also from Behring's "Die Wertbestimmung des Tetanus-antitoxins und seine Verwendung in der Menschenärztlichen und thierärztlichen Praxis." Deut. med. Woch., vol. 26, 1900, p. 29-52, etc.

A guinea pig is then injected subcutaneously with 10 c. c. of the serum in order to determine the presence of tetanus. If the guinea pig remains well, it is considered definite proof that the serum is free from this dangerous contamination. For this test it is necessary to use healthy, normal animals that have not been previously used for other purposes, so as to avoid the serum reaction.[a]

The official testing of antitoxin for potency at Ehrlich's institute is based entirely upon von Behring's method.[b] Von Behring proceeds as follows:

He dilutes the serum to be tested with physiological salt solution in the proportion of 1 c. c. of serum to 99 c. c. of the salt solution. One cubic centimeter of this dilution is then placed in an Erlenmeyer flask containing 38 c. c. of distilled water, and 1 c. c. of a high-grade test poison is added (the test poison No. $5 = \frac{N}{10}$). A uniform mixture of these three substances is obtained by shaking and the mixture is then allowed to stand thirty minutes, when a mouse of medium weight is injected subcutaneously with 0.4 c. c. If the mouse receiving 0.4 c. c. of this mixture remains well, then the serum contains at least 10 immunity units in 1 c. c., because 1 c. c. of the dilution 1:100 was entirely neutralized with 1 c. c. of the toxine, representing 0.1 normal poison. If the mouse after the subcutaneous inoculation of 0.4 c. c. of the mixture becomes tetanic, then the serum naturally contains somewhat less than 10 immunity units. If the mouse dies, we may form some judgment from the time intervening between the injection and death as to the approximate value of the serum.

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[a] Otto, R.: Das Theobald Smithsche Phänomen der Serum-Ueberempfindlichkeit. Leuthold-Gedenkschrift, Bd. 1.

Rosenau, M. J., and Anderson, John F.: A study of the cause of sudden death following the injection of horse serum. Bull. No. 29, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Wash., 1906.

Rosenau, M. J., and Anderson, John F.: Studies upon hypersusceptibility and immunity. Bull. No. 36, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Wash., 1907.

[b] Behring and Knorr: Ueber den Immunisierungswert und Heilwert des Tetanusheilserums bei weissen Mäusen. Zeit. f. Hyg., vol. 13, 1893.

v. Behring: Allg. Therapie der Infektionskrankheiten. Berlin and Vienna, Urban & Schwarzenberg, 1899.

———— Die Wertbestimmung des Tetanusantitoxins und seine Verwendung in der menschenarztlichen und tierärztlichen Praxis. Deut. med. Woch., 1900, No. 2.

More precise determination of the potency of serums injected into mice which remain entirely well after the injection of 0.4 c. c. of the mixture is made by further experiments. If in this way we obtain the L^0 value of the serum, we are enabled by a simple process of calculation to determine the antitoxic worth of the serum. That quantity of serum in 0.4 c. c. of the mixture containing 0.01 c. c. of test poison No. 5 (=L^0 dose) contains exactly $\frac{1}{1000}$ of an immunity unit.

Behring's notations, generally used in the German literature, are as follows:

1+Ms represents the fatal dose for 1 gram of mouse.
1+M represents the fatal dose for 1 gram of guinea pig.
Ms^{15} represents 15 times the minimal lethal dose for 1 gram of mouse.
The toxine is always represented by the plus sign, the antitoxin by the minus sign.

The present modification of the methods for testing tetanus antitoxin at Ehrlich's institute are largely based upon the work of Marx,[a] who found that the tetanus toxine could be preserved in a stable condition in a dried state in Ehrlich vacuum tubes.

The official testing of antitoxin is carried out in the following manner: One of the vacuum tubes containing the poison and another containing the serum are carefully opened by means of a file, and a certain quantity of water, say 5 c. c., added to each tube from a graduated pipette. The tubes are then carefully closed with a burnt cork. Each tube—the one containing toxine and the other serum—is now shaken many times in order to insure the complete solution of its contents. The contents of each tube are next emptied and carefully washed out with the remaining quantity of the diluting fluid. The serum must be so diluted that 1 c. c. of the solution represents just 0.01 of an immunity unit. After this has been accomplished the mixture of the toxine and antitoxin *in vitro* is made. For this purpose two series of eight flasks each are necessary. One c. c. of the standard serum solution is placed in each flask of the first series; 1 c. c. of a dilution of the unknown serum to be tested is placed in each flask of the second series. To each series is now added the following test doses of toxine, varying in accordance with the toxine from 0.8 to 1.5 c. c. These doses are so calculated that all the values from L^0

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[a] Marx, E.: Mitteilungen aus der prüfungstechnischen Praxis. Festschrift f. Robert Koch. Jena, Gustav Fischer, 1903.

———: Diagnostik, Serumtherapie und Prophylaxe. Bibliothek v. Coler, vol. 11, Berlin, 1902.

Dönitz, W.: Die Wertbemessung der Schutz- und Heilsera. Handbuch Kolle-Wassermann, vol. 4, part 2.

to L+ will be found in the series. Water is now added to the toxine-antitoxin mixtures, so that each flask will contain a total of 4 c. c. These flasks are well corked and remain one-half hour at room temperature protected from daylight by means of lead shells. After this period white mice are inoculated. Corresponding to the mixtures made, two series of eight mice each are injected. Each mouse receives 0.4 c. c. of the particular mixture under the skin of the right hind foot. Each mouse, therefore, receives $\frac{1}{1000}$ of an immunity unit plus varying amounts of toxine. As the doses of the toxine are selected so that each series of eight mice will give all values from the L° to the L+ limits, it is possible to determine from such a series all phases, as some animals will remain normal, others show symptoms of tetanus, and others die acutely.

If the unknown serum which is being tested has just the strength supposed for it in accordance with the above test, then the results in the two series of animals correspond precisely. If, however, it is weaker, there is a considerable difference in the results; from which it is easy to see about how much weaker the serum is than the standard serum. The following tables gives an example of tests made in accordance with the above explanation:

THE STATE TESTING OF THERAPEUTIC SERA.

Date: February 1, 1905.

Tetanus antitoxin: Manufacturer, X; No. y.

Sent in: January 31, 1905. Received: February 1, 1905.

Horses Nos. 4 and 6: Bled September 22 and 24, 1904. Quantity of serum sent in: 5 liters. Phenol content: 0.5 per cent. Strength claimed: 6 × normal.

Test for sterility: Sterile.

Test for phenol content: Mouse showed slight trembling.

Potency determination: 6 immunity units.

I. Standard serum: Dilution, 0.006 to 26 c. c.

II. Serum: X No. 11. Dilution, 1:600 (=1+19. 1+29, etc.).

III. Normal poison solution: To 1 c. c. of the diluted serum is added:

Poison.	Water.
0.8 c. c.	2.2 c. c.=4 c. c.
0.9 c. c.	2.1 c. c.=4 c.c.
1.0 c. c.	2.0 c. c.=4 c. c.
1.1 c. c.	1.9 c. c.=4 c. c.
1.2 c. c.	1.8 c. c.=4 c. c.
1.3 c. c.	1.7 c. c.=4 c. c.
1.4 c. c.	1.6 c. c.=4 c. c.
1.5 c. c.	1.5 c. c.=4 c. c.

The mixture of toxine and serum is allowed to stand half an hour at room temperature.

Quantity injected: 0.4 c. c.

Kind of animal: White mice (15 grams).

Kind of serum and date of test.	Result.	Kind of serum and date of test.	Result.
Standard serum.		*Serum No. y.*	
Mouse 1; poison, 0.8 c. c.; yellow head:		Mouse 9; poison, 0.8 c. c.; red head:	
February 2, 1905	0	February 2, 1905	0
February 3, 1905	0	February 3, 1905	0
February 4, 1905	0	February 4, 1905	0
February 5, 1905	0	February 5, 1905	0
February 6, 1905	0	February 6, 1905	0
February 7, 1905	0	February 7, 1905	0
Mouse 2; poison, 0.9 c. c.; yellow neck:		Mouse 10; poison, 0.9 c. c.; red neck:	
February 2, 1905	0	February 2, 1905	0
February 3, 1905	0	February 3, 1905	0
February 4, 1905	0(?)	February 4, 1905	0(?)
February 5, 1905	?	February 5, 1905	?
February 6, 1905	?	February 6, 1905	?
February 7, 1905	?	February 7, 1905	?
Mouse 3; poison, 1.0 c. c.; yellow back:		Mouse 11; poison, 1.0 c. c.; red back:	
February 2, 1905	?	February 2, 1905	?
February 3, 1905	Slight symptoms.	February 3, 1905	Slight symptoms.
February 4, 1905	Do.	February 4, 1905	Do.
February 5, 1905	Mild symptoms.	February 5, 1905	Mild symptoms.
February 6, 1905	Do.	February 6, 1905	Do.
February 7, 1905	Marked symptoms.	February 7, 1905	Marked symptoms.
Mouse 4; poison, 1.1 c. c.; yellow rump:		Mouse 12; poison, 1.1 c. c.; red rump:	
February 2, 1905	Slight symptoms.	February 2, 1905	Slight symptoms.
February 3, 1905	Mild symptoms.	February 3, 1905	Mild symptoms.
February 4, 1905	Marked symptoms.	February 4, 1905	Marked symptoms.
February 5, 1905	Do.	February 5, 1905	Do.
February 6, 1905	Do.	February 6, 1905	Do.
February 7, 1905	Very marked symptoms.	February 7, 1905	Very marked symptoms.
Mouse 5; poison, 1.2 c. c.; yellow right front foot:		Mouse 13; poison, 1.2 c. c.; red right front foot:	
February 2, 1905	Mild symptoms.	February 2, 1905	Mild symptoms.
February 3, 1905	Marked symptoms.	February 3, 1905	Marked symptoms.
February 4, 1905	Very marked symptoms.	February 4, 1905	Very marked symptoms.
February 5, 1905	Marked symptoms.	February 5, 1905	Marked symptoms.
February 6, 1905	Very severe symptoms.	February 6, 1905	Very severe symptoms.
February 7, 1905	Severe symptoms.	February 7, 1905	Severe symptoms.
Mouse 6; poison, 1.3 c. c.; yellow left front foot:		Mouse 14; poison, 1.3 c. c.; red left front foot:	
February 2, 1905	Mild symptoms.	February 2, 1905	Mild symptoms.
February 3, 1905	Very marked symptoms.	February 3, 1905	Very marked symptoms.
February 4, 1905	Marked symptoms.	February 4, 1905	Marked symptoms.
February 5, 1905	Very severe symptoms.	February 5, 1905	Very severe symptoms.
February 6, 1905	Severe symptoms.	February 6, 1905	Severe symptoms.
February 7, 1905	Violent tetanus.	February 7, 1905	Violent tetanus.

Kind of serum and date of test.	Result.	Kind of serum and date of test.	Result.
Standard serum—Cont'd.		*Serum No. y*—Cont'd.	
Mouse 7; poison, 1.4 c. c.; yellow right hind foot:		Mouse 15; poison, 1.4 c. c.; red right hind foot:	
February 2, 1905......	Marked symptoms.	February 2, 1905......	Marked symptoms.
February 3, 1905......	Very severe symptoms.	February 3, 1905......	Very severe symptoms.
February 4, 1905......	Tetany.	February 4, 1905......	Tetany.
February 5, 1905......	Dead, 4.	February 5, 1905......	Dead, 4.
Mouse 8; poison, 1.5 c. c.; yellow left hind foot:		Mouse 16; poison, 1.5 c. c.; red left hind foot:	
February 2, 1905......	Very severe symptoms.	February 2, 1905......	Very severe symptoms.
February 3, 1905......	Tetanus.	February 3, 1905......	Tetanus.
February 4, 1905......	Dead, 3.	February 4, 1905......	Dead, 3.

In the above illustrated case the two series of animals reacted precisely the same. It therefore shows that the antitoxin tested was in reality six times the normal strength.

It is evident, however, that the standardization of tetanus antitoxin by the German method is much more complicated than the testing of diphtheria antitoxin.[a]

The following preparations of tetanus antitoxin are found upon the German market (1904):

1. V. Behring's tetanus curative serum, made by Professor v. Behring, officially tested by Professor Ehrlich at the Frankfurt Institut for experimentelle Therapie. It is sold by the Marbourg firm of Doctor Siebert and Doctor Ziegenbein. The preparation is sold in two amounts, the one containing 100 immunity units (the curative dose) and the other 20 immunity units (for prophylactic use). Small vials of dried antitoxin, containing 20 immunity units, for dusting wounds, are also sold.

2. Tizzoni's tetanus serum, made by Tizzoni and Cattani. This is sold by the firm of Merck, in Darmstadt. The preparation is sold in original vials of 5 grams each (normal dose) = 5,000,000 immunity units.

THE ITALIAN METHOD OF STANDARDIZING TETANUS ANTITOXIN.

The Tizzoni preparation is now sold by Merck, at Darmstadt, and is said to contain in every cubic centimeter 80,000 immunity units (I. U.); in other words, the antitoxin amount which will neutralize 80,000 toxine units. The toxine unit, according to this reckoning, is that amount of toxine which will kill a rabbit weighing 1 kilogram in from four to five days. The dried antitoxin is usually furnished, 0.1 of a gram of which represents 1 c. c. of serum.

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[a] V. Lingelsheim: Immunität bei Tetanus. Kolle & Wassermann's Handbuch, vol. 4, part 2.

The Italian method is based entirely upon the work and theoretic considerations of Prof. Guido Tizzoni[a] and Giuseppina Cattani.

Tizzoni early abandoned the methods of standardizing tetanus antitoxin by injecting the serum and the poison separately into the animal with an interval of twenty-four hours. He soon found that the method of Ehrlich, Kossel, and Wassermann, which consists of injections of mixtures *in vitro* of toxine and antitoxin (of diphtheria), gives more accurate and reliable results. In applying this method to tetanus, Tizzoni encountered certain difficulties, due more particularly to the unstable nature of the tetanus toxine. He correctly observes that "Nothing more embarrassing can be imagined than experimenting with a toxine which changes in the hands of the experimenter and the precise grade of the potency of which can not be known at a given moment. Researches pursued under these conditions are long, laborious, often contradictory, uncertain, and confused; the conclusions derived from them are rather deductions than demonstrations; it is impossible to institute a continuous series of experiments, and the few certain results obtained are arrived at almost by accident in the midst of a farrago of observations which are of no service on account of the defects, in greater or less degree, in the dose of toxine used." A stable toxine from tetanus cultures can not be obtained. Cultures reach their maximum toxicity from the seventh to the fifteenth day and then gradually weaken, even when kept cold and protected from light. Filtration weakens the poison. Tizzoni found that filtrates even when kept under oil, in vacuo, or under gases lose their toxic value.

Tizzoni and Kitasato independently found that if filtered cultures are reduced to dryness in vacuo at a low temperature (20°–22° C.) the resulting yellow-brown scales gradually lose toxicity. No better success was had with Brieger and Frankel's method of precipitation with alcohol. It seems that Tizzoni was the first to obtain a precipitated tetanus toxine with ammonium sulphate, following the method of Brieger and Frankel with other toxines. Buchner and Rapp used the same method and obtained a stable poison. But Tizzoni found these precipitates not to be constant. He believes that these precipitates first decompose rapidly and then very slowly, so that they contain a large quantity of transformed toxine, capable of confusing the results of experiments.

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[a] The information in regard to the Italian method of standardizing tetanus antitoxin was gotten from "Sul Modo di Determinare la Potenza del Sero Antitetanico, col metodo della mescolanza in vitro" by Prof. Guido Tizzoni, in "Memorie della r. Accademia delle Scienze dell' Instituto di Bologna," Tomo 8, Serie Quinta, Bologna, 1899–1900. Professor Tizzoni's entire article was kindly translated for us by Miss E. R. Jones.

Tizzoni believes that the weakening of the dried precipitated toxin is due to the production of an acid substance. It was for this reason that Knorr dissolved the precipitate in a 10 per cent solution of sodium chloride.

Tizzoni at one time believed the instability of the precipitated toxine to be due to the ammonium sulphate which remained in the precipitate and eliminated this by spreading the fresh moist precipitate upon porous porcelain; but the toxine so obtained showed itself to be no more stable than those that preceded it. On the other hand, he obtained very satisfactory results by the elimination of the sulphate of ammonium by means of dialysis. Tizzoni claims that tetanus toxine does not dialyze. He recognizes that the stability of the toxine so prepared is not indefinite, for he states that this dialyzed precipitate passes through some modification in the course of time. The toxine, however, is sufficiently stable for the purpose of standardizing antitoxin and for scientific tests. Besides serving for the preparation of the standard antitoxin, this method is applicable for concentrating the poison for injection into animals. Tizzoni showed further that almost all of the toxines produced by a culture are precipitated by ammonium sulphate—that is, there is very little loss in this process.

Tizzoni found that antitoxic serums measured against fresh tetanus toxine and an old and weakened poison gave different results. He believes that this is due largely to the modified toxine or "toxoid" contained in the weakened serum.

Tizzoni considers it of importance to use for the preparation of the toxine, cultures which at the period of their greatest development contain the greatest degree of toxicity. Thus 0.001 c. c. of the filtrate should constantly kill 1 kilogram of rabbit in four days. Cultures which at the moment of precipitation with sulphate of ammonium do not possess this toxicity are rejected.

In view of the differences shown by different species of animals Tizzoni always uses the rabbit for standardizing both toxine and antitoxin, and finds it necessary to take into account the weight of the animal. He concluded that the principal condition to be realized in the standardizing of tetanus antitoxin is the possession of a stable toxine. He dwells upon the importance of determining the therapeutic value of the serums in relation to the antitoxic value as determined by animal tests. Too much confusion prevails to-day in the method of representing the value of tetanus antitoxic serums. This condition should be terminated by adopting a language common to all and easily understood. In the present state of affairs the confusion has arrived at such a point that anyone concerned in a discussion can not judge from the value attributed to two serums of different origin the real relation of their potencies. Tizzoni therefore pleads for a simplification and unification of methods, and commends his own for general use. Tizzoni prepares two solutions—one of toxine and one of serum—the first in distilled water and the second in 0.75 per cent salt solution. The two solutions are mixed, and after one-half hour of contact the entire mixture is injected into the posterior part of the thigh of a rabbit weighing about 1 kilogram. The value of the serum is always calculated upon the titre of the mixture which does not cause symptoms in an animal.

THE FRENCH METHOD OF STANDARDIZING TETANUS ANTITOXIN.

The strength of the tetanus antitoxin manufactured by the Pasteur Institute is not stated upon the packages sold by the Pasteur Vaccine Company. The prophylactic dose is given as 10 c. c. It is stated that the antitoxic dose of the serum amounts to 1,000,000,000; that is, to protect a mouse against the smallest fatal dose it is sufficient to use $\frac{1}{1,000,000,000}$ of its weight of serum.

The description of the method used in France was kindly sent to us in a personal communication by Professor Arloing, of the Bacteriological Institute of Lyons, having been prepared for him by Professor Courmont, who has charge of the control of antitetanic serum in that institute.

A number of guinea pigs are injected under the skin with quantities of serum equal to $\frac{1}{500,000}$, $\frac{1}{1,000,000}$, $\frac{1}{10,000,000}$, $\frac{1}{10,000,000}$, and even $\frac{1}{1,000,000,000}$ of their weight. Twelve to twenty hours afterwards they, as well as the controls, are inoculated subcutaneously with a single fatal dose of tetanus toxine. The time of death of those which develop tetanus is noted. The serum is then said to have an immunizing or antitoxic power of $\frac{1}{500,000}$, $\frac{1}{1,000,000}$, etc., according as the guinea pig which received $\frac{1}{500,000}$, $\frac{1}{1,000,000}$, etc., of its weight of serum has survived the injection of tetanus toxine fatal for the control.

Professor Courmont adds that their serum is usually of a strength of $\frac{1}{1,000,000}$ and never goes below $\frac{1}{500,000}$.

The French antitoxic serums are made at the Institut Pasteur, Paris, and l'Institut Bacteriologique, Lyon.

TABLES FOR DILUTING THE TOXINE AND ANTITOXIN.

The following tables are used for diluting the tetanus toxines and antitoxins in this laboratory. They are published not only because they will save time and the possibility of error incident to such calculations, but because it is of some importance that the same methods of dilution be used by different laboratories in order that the results may be strictly comparable. This apparently trifling matter is of some importance in tetanus work on account of the known influence of concentration upon the tetanus poison and its antibody.

TABLE No. 8.

A.— 1+99=1:100 1 c. c.=0.01
B. (1 of A)+9=1:1000 1 c. c.=0.001

0.7 c. c. of B=0.0007 or 1/1,430
.8 c. c. of B= .0008 or 1/1,250
.9 c. c. of B= .0009 or 1/1,111
1.0 c. c. of B= .0010 or 1/1,000
1.1 c. c. of B= .0011 or 1/909
1.2 c. c. of B= .0012 or 1/833
1.3 c. c. of B= .0013 or 1/769
1.4 c. c. of B= .0014 or 1/714
1.5 c. c. of B= .0015 or 1/666
1.6 c. c. of B= .0016 or 1/625
1.7 c. c. of B= .0017 or 1/588
1.8 c. c. of B= .0018 or 1/555
1.9 c. c. of B= .0019 or 1/526
2.0 c. c. of B= .0020 or 1/500
2.1 c. c. of B= .0021 or 1/476
2.2 c. c. of B=0.0022 or 1/454
2.3 c. c. of B= .0023 or 1/435
2.4 c. c. of B= .0024 or 1/417
2.5 c. c. of B= .0025 or 1/400
2.6 c. c. of B= .0026 or 1/384
2.7 c. c. of B= .0027 or 1/370
2.8 c. c. of B= .0028 or 1/357
2.9 c. c. of B= .0029 or 1/345
3.0 c. c. of B= .0030 or 1/333
3.1 c. c. of B= .0031 or 1/322
3.2 c. c. of B= .0032 or 1/312
3.3 c. c. of B= .0033 or 1/303
3.4 c. c. of B= .0034 or 1/294
3.5 c. c. of B= .0035 or 1/285

TABLE No. 9.

A. 1+99=1:100 1 c. c.=0.01
B. (1 of A)+19=1:2000 1 c. c.=0.0005

0.7 c. c. of B=0.00035 or 1/2,860
.8 c. c. of B= .00040 or 1/2,500
.9 c. c. of B= .00045 or 1/2,222
1.0 c. c. of B= .00050 or 1/2,000
1.1 c. c. of B= .00055 or 1/1,818
1.2 c. c. of B= .00060 or 1/1,666
1.3 c. c. of B= .00065 or 1/1,538
1.4 c. c. of B= .00070 or 1/1,428
1.5 c. c. of B= .00075 or 1/1,333
1.6 c. c. of B= .00080 or 1/1,250
1.7 c. c. of B= .00085 or 1/1,174
1.8 c. c. of B= .00090 or 1/1,111
1.9 c. c. of B= .00095 or 1/1,057
2.0 c. c. of B= .00100 or 1/1,000
2.1 c. c. of B= .00105 or 1/952
2.2 c. c. of B=0.00110 or 1/909
2.3 c. c. of B= .00115 or 1/869
2.4 c. c. of B= .00120 or 1/833
2.5 c. c. of B= .00125 or 1/800
2.6 c. c. of B= .00130 or 1/769
2.7 c. c. of B= .00135 or 1/741
2.8 c. c. of B= .00140 or 1/714
2.9 c. c. of B= .00145 or 1/689
3.0 c. c. of B= .00150 or 1/666
3.1 c. c. of B= .00155 or 1/645
3.2 c. c. of B= .00160 or 1/625
3.3 c. c. of B= .00165 or 1/606
3.4 c. c. of B— .00170 or 1/588
3.5 c. c. of B= .00175 or 1/571

TABLE No. 10.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+29=1:3000 1 c. c.=0.00033

0.7 c. c. of B=0.00024 or 1/4,170
.8 c. c. of B= .00027 or 1/3,700
.9 c. c. of B= .00030 or 1/3,333
1.0 c. c. of B= .000333333 or 1/3,000
1.1 c. c. of B= .00036 or 1/2,777
1.2 c. c. of B= .00040 or 1/2,500
1.3 c. c. of B= .00043 or 1/2,302
1.4 c. c. of B= .00046 or 1/2,173
1.5 c. c. of B= .00050 or 1/2,000
1.6 c. c. of B= .00053 or 1/1,850
1.7 c. c. of B= .00056 or 1/1,786
1.8 c. c. of B= .00060 or 1/1,666
1.9 c. c. of B= .00063 or 1/1,587
2.0 c. c. of B= .00066 or 1/1,515
2.1 c. c. of B= .00070 or 1/1,428
2.2 c. c. of B=0.00073 or 1/1,369
2.3 c. c. of B= .00076 or 1/1,304
2.4 c. c. of B= .00080 or 1/1,250
2.5 c. c. of B= .00083 or 1/1,200
2.6 c. c. of B= .00086 or 1/1,154
2.7 c. c. of B= .00090 or 1/1,111
2.8 c. c. of B= .00093 or 1/1,071
2.9 c. c. of B= .00096 or 1/1,034
3.0 c. c. of B= .00100 or 1/1,000
3.1 c. c. of B= .00103 or 1/ 967
3.2 c. c. of B= .00107 or 1/ 937
3.3 c. c. of B= .00110 or 1/ 909
3.4 c. c. of B= .00113 or 1/ 882
3.5 c. c. of B= .00116 or 1/ 857

TABLE No. 11.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+39=1:4000 1 c. c.=0.00025

0.7 c. c. of B=0.000175 or 1/5,714
.8 c. c. of B= .000200 or 1/5,000
.9 c. c. of B= .000225 or 1/4,444
1.0 c. c. of B= .000250 or 1/4,000
1.1 c. c. of B= .000275 or 1/3,636
1.2 c. c. of B= .000300 or 1/3,333
1.3 c. c. of B= .000325 or 1/3,077
1.4 c. c. of B= .000350 or 1/2,860
1.5 c. c. of B= .000375 or 1/2,666
1.6 c. c. of B= .000400 or 1/2,500
1.7 c. c. of B= .000425 or 1/2,353
1.8 c. c. of B= .000450 or 1/2,222
1.9 c. c. of B= .000475 or 1/2,105
2.0 c. c. of B= .000500 or 1/2,000
2.1 c. c. of B= .000525 or 1/1,905
2.2 c. c. of B=0.000550 or 1/1,818
2.3 c. c. of B= .000575 or 1/1,739
2.4 c. c. of B= .000600 or 1/1,666
2.5 c. c. of B= .000625 or 1/1,600
2.6 c. c. of B= .000650 or 1/1,538
2.7 c. c. of B= .000675 or 1/1,481
2.8 c. c. of B= .000700 or 1/1,428
2.9 c. c. of B= .000725 or 1/1,379
3.0 c. c. of B= .000750 or 1/1,333
3.1 c. c. of B= .000775 or 1/1,290
3.2 c. c. of B= .000800 or 1/1,250
3.3 c. c. of B= .000825 or 1/1,212
3.4 c. c. of B= .000850 or 1/1,174
3.5 c. c. of B= .000875 or 1/1,143

TABLE No. 12.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A) +49=1:5000 1 c. c.=0.0002

0.7 c. c. of B=0.00014 or 1/7,143
.8 c. c. of B= .00016 or 1/6,250
.9 c. c. of B= .00018 or 1/5,555
1.0 c. c. of B= .00020 or 1/5,000
1.1 c. c. of B= .00022 or 1/4,545
1.2 c. c. of B= .00024 or 1/4,170
1.3 c. c. of B= .00026 or 1/3,846
1.4 c. c. of B= .00028 or 1/3,571
1.5 c. c. of B= .00030 or 1/3,333
1.6 c. c. of B= .00032 or 1/3,125
1.7 c. c. of B= .00034 or 1/2,941
1.8 c. c. of B= .00036 or 1/2,777
1.9 c. c. of B= .00038 or 1/2,631
2.0 c. c. of B= .00040 or 1/2,500
2.1 c. c. of B= .00042 or 1/2,381
2.2 c. c. of B=0.00044 or 1/2,272
2.3 c. c. of B= .00046 or 1/2,173
2.4 c. c. of B= .00048 or 1/2,083
2.5 c. c. of B= .00050 or 1/2,000
2.6 c. c. of B= .00052 or 1/1,923
2.7 c. c. of B= .00054 or 1/1,851
2.8 c. c. of B= .00056 or 1/1,786
2.9 c. c. of B= .00058 or 1/1,724
3.0 c. c. of B= .00060 or 1/1,666
3.1 c. c. of B= .00062 or 1/1,613
3.2 c. c. of B= .00064 or 1/1,562
3.3 c. c. of B= .00066 or 1/1,515
3.4 c. c. of B= .00068 or 1/1,470
3.5 c. c. of B= .00070 or 1/1,428

TABLE No. 13.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A) +59=1:6000 1 c.c.=0.00017

0.7 c. c. of B=0.000117 or 1/8,571
.8 c. c. of B= .000133 or 1/7,500
.9 c. c. of B= .000150 or 1/6,666
1.0 c. c. of B= .000166666 or 1/6,000
1.1 c. c. of B= .000183 or 1/5,454
1.2 c. c. of B= .000199 or 1/5,000
1.3 c. c. of B= .000216 or 1/4,615
1.4 c. c. of B= .000233 or 1/4,285
1.5 c. c. of B= .000249 or 1/4,000
1.6 c. c. of B= .000266 or 1/3,750
1.7 c. c. of B= .000283 or 1/3,529
1.8 c. c. of B= .000300 or 1/3,333
1.9 c. c. of B= .000317 or 1/3,158
2.0 c. c. of B= .000333 or 1/3,000
2.1 c. c. of B= .000350 or 1/2,857
2.2 c. c. of B=0.000367 or 1/2,727
2.3 c. c. of B= .000383 or 1/2,609
2.4 c. c. of B= .000400 or 1/2,500
2.5 c. c. of B= .000417 or 1/2,400
2.6 c. c. of B= .000433 or 1/2,307
2.7 c. c. of B= .00045 or 1/2,222
2.8 c. c. of B= .000467 or 1/2,143
2.9 c. c. of B= .000483 or 1/2,069
3.0 c. c. of B= .000500 or 1/2,000
3.1 c. c. of B= .000517 or 1/1,938
3.2 c. c. of B= .000533 or 1/1,875
3.3 c. c. of B= .000550 or 1/1,818
3.4 c. c. of B= .000567 or 1/1,764
3.5 c. c. of B= .000583 or 1/1,714

TABLE No. 14.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+69=1:7000 1 c. c.=0.000142857

0.7 c. c. of B=0.000100 or 1/10, 000
.8 c. c. of B= .000114 or 1/8, 750
.9 c. c. of B= .000129 or 1/7, 777
1.0 c. c. of B= .000142857 or 1/7, 000
1.1 c. c. of B= .000157 or 1/6, 363
1.2 c. c. of B= .000171 or 1/5, 833
1.3 c. c. of B= .000185 or 1/5, 385
1.4 c. c. of B= .000200 or 1/5, 000
1.5 c. c. of B= .000214 or 1/4, 666
1.6 c. c. of B= .000229 or 1/4, 375
1.7 c. c. of B= .000243 or 1/4, 118
1.8 c. c. of B= .000257 or 1/3, 888
1.9 c. c. of B= .000271 or 1/3, 684
2.0 c. c. of B= .000285 or 1/3, 500
2.1 c. c. of B= .000300 or 1/3, 333
2.2 c. c. of B=0.000314 or 1/3, 181
2.3 c. c. of B= .000329 or 1/3, 043
2.4 c. c. of B= .000343 or 1/2, 916
2.5 c. c. of B= .000357 or 1/2, 800
2.6 c. c. of B= .000371 or 1/2, 692
2.7 c. c. of B= .000385 or 1/2, 592
2.8 c. c. of B= .000400 or 1/2, 500
2.9 c. c. of B= .000414 or 1/2, 414
3.0 c. c. of B= .000429 or 1/2, 333
3.1 c. c. of B= .000443 or 1/2, 258
3.2 c. c. of B= .000457 or 1/2, 187
3.3 c. c. of B= .000471 or 1/2, 121
3.4 c. c. of B= .000485 or 1/2, 059
3.5 c. c. of B= .000500 or 1/2, 000

TABLE No. 15.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+79=1:8000 1 c. c.=0.000125

0.7 c. c. of B=0.0000875 or 1/11, 428
.8 c. c. of B= .0001000 or 1/10, 000
.9 c. c. of B= .0001125 or 1/8, 888
1.0 c. c. of B= .0001250 or 1/8, 000
1.1 c. c. of B= .0001375 or 1/7, 272
1.2 c. c. of B= .0001500 or 1/6, 666
1.3 c. c. of B= .0001625 or 1/6, 154
1.4 c. c. of B= .0001750 or 1/5, 714
1.5 c. c. of B= .0001875 or 1/5, 333
1.6 c. c. of B= .0002000 or 1/5, 000
1.7 c. c. of B= .0002125 or 1/4, 706
1.8 c. c. of B= .0002250 or 1/4, 444
1.9 c. c. of B= .0002375 or 1/4, 210
2.0 c. c. of B= .0002500 or 1/4, 000
2.1 c. c. of B= .0002625 or 1/3, 809
2.2 c. c. of B=0.0002750 or 1/3, 636
2.3 c. c. of B= .0002875 or 1/3, 478
2.4 c. c. of B= .0003000 or 1/3, 333
2.5 c. c. of B= .0003125 or 1/3, 200
2.6 c. c. of B= .0003250 or 1/3, 077
2.7 c. c. of B= .0003375 or 1/2, 962
2.8 c. c. of B= .0003500 or 1/2, 860
2.9 c. c. of B= .0003625 or 1/2, 758
3.0 c. c. of B= .0003750 or 1/2, 666
3.1 c. c. of B= .0003875 or 1/2, 581
3.2 c. c. of B= .0004000 or 1/2, 500
3.3 c. c. of B= .0004125 or 1/2, 424
3.4 c. c. of B= .0004250 or 1/2, 353
3.5 c. c. of B= .0004375 or 1/2, 286

Table No. 16.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+89=1:9,000 1 c. c.=0.000111

0.7 c. c. of B=0.000077 or 1/12,859
.8 c. c. of B= .000088 or 1/11,250
.9 c. c. of B= .000100 or 1/10,000
1.0 c. c. of B= .000111111 or 1/9,000
1.1 c. c. of B= .000122 or 1/8,173
1.2 c. c. of B= .000133 or 1/7,500
1.3 c. c. of B= .000144 or 1/6,923
1.4 c. c. of B= .000155 or 1/6,428
1.5 c. c. of B= .000166 or 1/6,000
1.6 c. c. of B= .000177 or 1/5,625
1.7 c. c. of B= .000188 or 1/5,293
1.8 c. c. of B= .000200 or 1/5,000
1.9 c. c. of B= .000211 or 1/4,736
2.0 c. c. of B= .000222 or 1/4,500
2.1 c. c. of B= .000233 or 1/4,286
2.2 c. c. of B=0.000244 or 1/4,091
2.3 c. c. of B= .000255 or 1/3,913
2.4 c. c. of B= .000266 or 1/3,750
2.5 c. c. of B= .000277 or 1/3,600
2.6 c. c. of B= .000288 or 1/3,461
2.7 c. c. of B= .000300 or 1/3,333
2.8 c. c. of B= .000311 or 1/3,214
2.9 c. c. of B= .000322 or 1/3,103
3.0 c. c. of B= .000333 or 1/3,000
3.1 c. c. of B= .000344 or 1/2,903
3.2 c. c. of B= .000355 or 1/2,812
3.3 c. c. of B= .000366 or 1/2,727
3.4 c. c. of B= .000377 or 1/2,647
3.5 c. c. of B= .000388 or 1/2,571

Table No. 17.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+99=1:10,000 1 c. c.=0.0001

0.7 c. c. of B=0.00007 or 1/14,285
.8 c. c. of B= .00008 or 1/12,500
.9 c. c. of B= .00009 or 1/11,111
1.0 c. c. of B= .00010 or 1/10,000
1.1 c. c. of B= .00011 or 1/9,090
1.2 c. c. of B= .00012 or 1/8,333
1.3 c. c. of B= .00013 or 1/7,692
1.4 c. c. of B= .00014 or 1/7,143
1.5 c. c. of B= .00015 or 1/6,666
1.6 c. c. of B= .00016 or 1/6,250
1.7 c. c. of B= .00017 or 1/5,882
1.8 c. c. of B= .00018 or 1/5,555
1.9 c. c. of B=0.00019 or 1/5,263
2.0 c. c. of B= .00020 or 1/5,000
2.1 c. c. of B= .00021 or 1/4,762
2.2 c. c. of B= .00022 or 1/4,545
2.3 c. c. of B= .00023 or 1/4,348
2.4 c. c. of B= .00024 or 1/4,166
2.5 c. c. of B= .00025 or 1/4,000
2.6 c. c. of B= .00026 or 1/3,846
2.7 c. c. of B= .00027 or 1/3,703
2.8 c. c. of B= .00028 or 1/3,571
2.9 c. c. of B= .00029 or 1/3,448
3.0 c. c. of B= .00030 or 1/3,333

Table No. 18.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+19=1:20,000 1 c. c.=0.00005

0.7 c. c. of C=0.000035 or 1/28,571
.8 c. c. of C= .000040 or 1/25,000
.9 c. c. of C= .000045 or 1/22,222
1.0 c. c. of C= .000050 or 1/20,000
1.1 c. c. of C= .000055 or 1/18,181
1.2 c. c. of C= .000060 or 1/16,666
1.3 c. c. of C= .000065 or 1/15,385
1.4 c. c. of C= .000070 or 1/14,285
1.5 c. c. of C= .000075 or 1/13,333
1.6 c. c. of C= .000080 or 1/12,500
1.7 c. c. of C= .000085 or 1/11,764
1.8 c. c. of C= .000090 or 1/11,111
1.9 c. c. of C=0.000095 or 1/10,526
2.0 c. c. of C= .000100 or 1/10,000
2.1 c. c. of C= .000105 or 1/9,524
2.2 c. c. of C= .000110 or 1/9,090
2.3 c. c. of C= .000115 or 1/8,695
2.4 c. c. of C= .000120 or 1/8,333
2.5 c. c. of C= .000125 or 1/8,000
2.6 c. c. of C= .000130 or 1/7,615
2.7 c. c. of C= .000135 or 1/7,407
2.8 c. c. of C= .000140 or 1/7,143
2.9 c. c. of C= .000145 or 1/6,896
3.0 c. c. of C= .000150 or 1/6,666

TABLE No. 19.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+29=1:30,000 1 c. c.=0.0000333

0.7 c. c. of C=0.0000233 or 1/42,857	1.9 c. c. of C=0.0000633 or 1/15,799
.8 c. c. of C= .0000266 or 1/37,500	2.0 c. c. of C= .0000666 or 1/15,000
.9 c. c. of C= .00003 or 1/33,333	2.1 c. c. of C= .00007 or 1/14,285
1.0 c. c. of C= .0000333 or 1/30,000	2.2 c. c. of C= .0000733 or 1/13,642
1.1 c. c. of C= .0000366 or 1/37,322	2.3 c. c. of C= .0000766 or 1/13,055
1.2 c. c. of C= .00004 or 1/25,000	2.4 c. c. of C= .00008 or 1/12,500
1.3 c. c. of C= .0000433 or 1/23,095	2.5 c. c. of C= .0000833 or 1/12,000
1.4 c. c. of C= .0000466 or 1/21,428	2.6 c. c. of C= .0000866 or 1/11,547
1.5 c. c. of C= .00005 or 1/20,000	2.7 c. c. of C= .00009 or 1/11,111
1.6 c. c. of C= .0000533 or 1/18,761	2.8 c. c. of C= .0000933 or 1/10,504
1.7 c. c. of C= .0000566 or 1/17,668	2.9 c. c. of C= .0000966 or 1/10,352
1.8 c. c. of C= .00006 or 1/16,666	3.0 c. c. of C= .0001 or 1/10,000

TABLE No. 20.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+39=1:40,000 1 c. c.=0.000025

0.7 c. c. of C=0.0000175 or 1/57,142	1.9 c. c. of C=0.0000475 or 1/21,053
.8 c. c. of C= .00002 or 1/50,000	2.0 c. c. of C= .00005 or 1/20,000
.9 c. c. of C= .0000225 or 1/44,444	2.1 c. c. of C= .0000525 or 1/19,048
1.0 c. c. of C= .000025 or 1/40,000	2.2 c. c. of C= .000055 or 1/18,181
1.1 c. c. of C= .0000275 or 1/36,363	2.3 c. c. of C= .0000575 or 1/17,391
1.2 c. c. of C= .00003 or 1/33,333	2.4 c. c. of C= .00006 or 1/16,666
1.3 c. c. of C= .0000325 or 1/30,769	2.5 c. c. of C= .0000625 or 1/16,000
1.4 c. c. of C= .000035 or 1/28,571	2.6 c. c. of C= .000065 or 1/15,384
1.5 c. c. of C= .0000375 or 1/26,666	2.7 c. c. of C= .0000675 or 1/14,814
1.6 c. c. of C= .00004 or 1/25,000	2.8 c. c. of C= .00007 or 1/14,285
1.7 c. c. of C= .0000425 or 1/23,529	2.9 c. c. of C= .0000725 or 1/13,793
1.8 c. c. of C= .000045 or 1/22,222	3.0 c. c. of C= .000075 or 1/13,333

TABLE No. 21.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+49=1:50,000 1 c. c.=0.00002

0.7 c. c. of C=0.000014 or 1/71,428	1.9 c. c. of C=0.000038 or 1/26,316
.8 c. c. of C= .000016 or 1/62,500	2.0 c. c. of C= .000040 or 1/25,000
.9 c. c. of C= .000018 or 1/55,555	2.1 c. c. of C= .000042 or 1/23,809
1.0 c. c. of C= .000020 or 1/50,000	2.2 c. c. of C= .000044 or 1/22,818
1.1 c. c. of C= .000022 or 1/45,454	2.3 c. c. of C= .000046 or 1/21,739
1.2 c. c. of C= .000024 or 1/41,666	2.4 c. c. of C= .000048 or 1/20,833
1.3 c. c. of C= .000026 or 1/38,431	2.5 c. c. of C= .000050 or 1/20,000
1.4 c. c. of C= .000028 or 1/35,714	2.6 c. c. of C= .000052 or 1/19,230
1.5 c. c. of C= .000030 or 1/33,333	2.7 c. c. of C= .000054 or 1/18,518
1.6 c. c. of C= .000032 or 1/31,250	2.8 c. c. of C= .000056 or 1/17,857
1.7 c. c. of C= .000034 or 1/29,412	2.9 c. c. of C= .000058 or 1/17,241
1.8 c. c. of C= .000036 or 1/27,777	3.0 c. c. of C= .000060 or 1/16,666

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

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No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

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No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species Paragordius varius), with summary of all cases reported to date. By Ch. Wardell Stiles.

No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

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No. 43.—The standardization of tetanus antitoxin. An American unit established under authority of the act of July 1, 1902. By M. J. Rosenau and John F. Anderson.

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